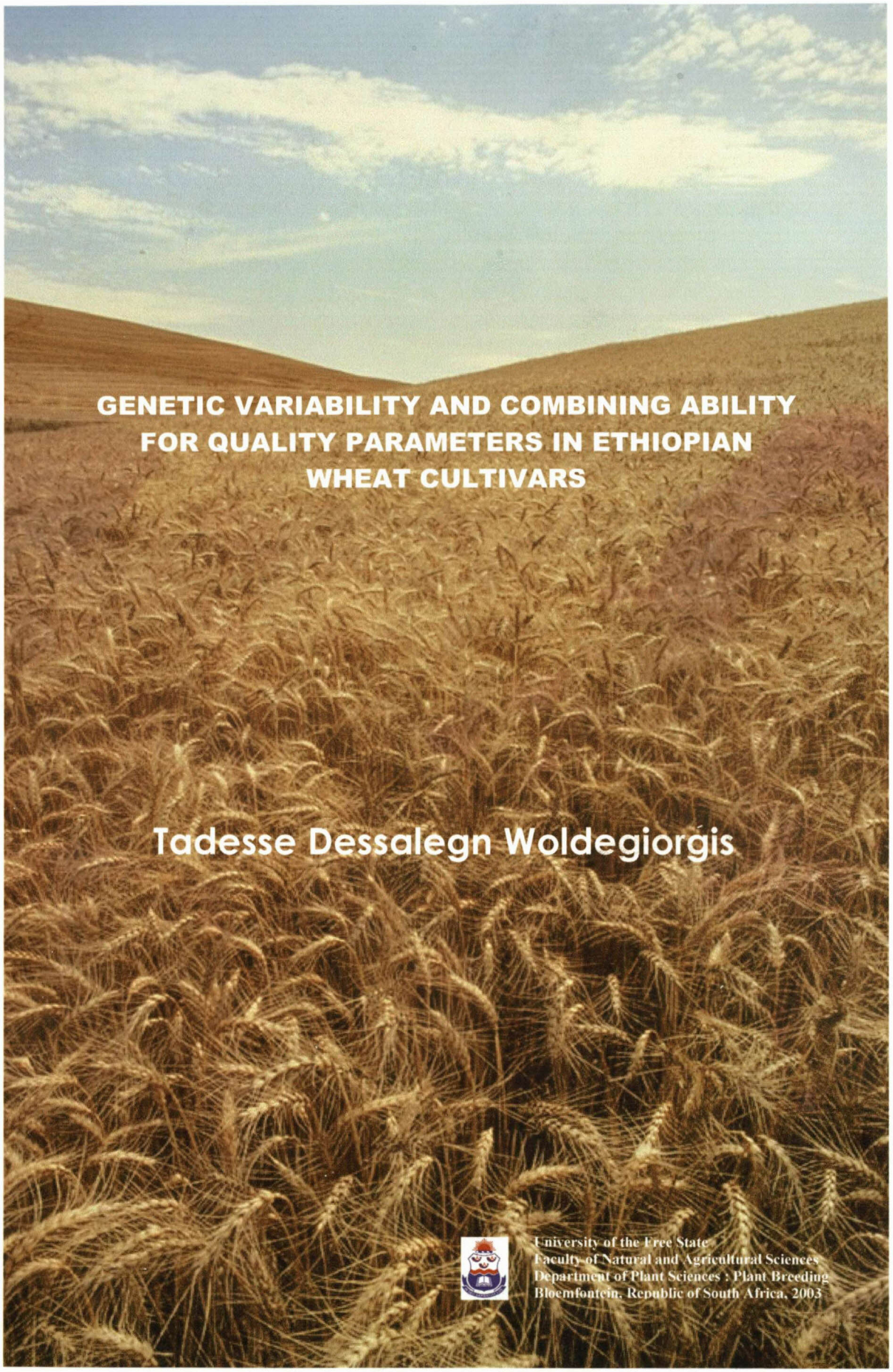


GENETIC VARIABILITY AND COMBINING ABILITY FOR QUALITY PARAMETERS IN ETHIOPIAN WHEAT CULTIVARS

Tadesse Dessalegn Woldegiorgis

University of the Free State
Faculty of Natural and Agricultural Sciences
Department of Plant Sciences : Plant Breeding
Bloemfontein, Republic of South Africa, 2003

Genetic variability and combining ability for quality parameters in Ethiopian wheat cultivars

by

Tadesse Dessalegn Woldegiorgis

A dissertation submitted in the fulfillment of the requirements for the degree of

Philosophiae Doctor

University of the Free State
Faculty of Natural and Agricultural Sciences
Department of Plant Sciences
Plant Breeding
Bloemfontein, Republic of South Africa, 2003

Major Promoter: Prof. C.S. van Deventer (Ph.D)

Co-Promotor : Prof. M.T. Labuschagne (Ph.D)

2003

Dedication

To my wife Yehuwalashet Feleke and our children, Barnabas, Kale and Kaleh

To my mother Sahlemariam Ayele and in memory of my Father, Dessalegn Woldegiorgis

"The roots of education are bitter, but the fruit is sweet"

-Aristotle

(Source (2003): http:/aclib.sabinet co.za)

DECLARATION

I hereby declare that this dissertation is submitted to the department of plant breeding at the University of the Free State in compliance with the requirements for the degree Philosophiae Doctor is my own exertion and has not been previously submitted to any other University. I concede that the University of the Free State has the copyright of this dissertation.

Signature: 

Tadesse Dessalegn

2003

Table of contents

List of figures

List of tables

ABBREVIATIONS

AACC..... American Association of Cereal Chemists

A-PAGE..... Acid Polyacrylamide Gel Electrophoresis

AWRC .....Alkaline Water Retention Capacity

BFLY.....Break Flour Yield

cov .....Covariance

CV .....Coefficient of Variation

Env .....Environment

FABS..... Farinograph Water Absorption

FCL .....Flour Color

FLN.....Falling Number

FLY.....Flour Yield

FPC..... Flour Protein Content

GCA.....General Combining Ability

Gli.....Gliadin

GLM.....General Linear Model

Glu.....Glutenin

GLUT..... Wet Gluten

GYD..... Grain Yield

H.....Heterosis

h^2_n .....Narrow sense heritability

HLW.....Hectoliter Weight

HMW-GS.....High Molecular Weight Glutenin Subunit

HP.....Better Parent

L.....Extensibility

LFV..... Bread Loaf Volume

LMW-GS..... Low Molecular Weight Glutenin Subunits

LSD..... Least Significant Differences

MA.....Mid-parent Advantage

MDT..... Mixograph Development Time

Me..... Error mean square

Mg.....Mean square of genotypes

M_i.....Mean square due to interaction;

MP.....Mid-parents

MS.....Mean Square

NCSS.....Number Cruncher Statistical System

P.....Tenacity

R^2.....Coefficient of determination

RCB..... Randomized Complete Block

r_g.....Genotypic correlations

RP-HPLC.....Reverse Phase High Performance Liquid Chromatography

SAS.....Statistical Analysis System

SCA.....Specific Combining Ability

SDS-PAGE..... Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis

SDSS..... Sodium Dodecyl Sulphate Sedimentation

SE.....Standard Error

SKCS.....Single Kernel Characteristics System

SKDM.....SKCS-Seed Diameter

SKHI.....SKCS- Hardness Index

SKWT.....SKCS-Seed Weight

TGW.....1000 Grains Weight

VIF.....Variance Inflation Factor

VK.....Vitreous Kernels

W.....Alveograph dough (gluten) strength

σ^2.....Variance

σ^2_e.....Error variance

σ^2_g.....Genotypic variance

σ^2_i.....Genotype by environment interaction variance

ϕ^2.....Variance due to fixed effect

σ^2_A.....Additive genetic variance

σ^2_{AE}.....Additive by environmental variance

σ^2_D.....Dominance genetic variance

σ^2_{DE}.....Dominance by environmental variance

ACKNOWLEDGEMENT

I am highly indebted to record my earnest gratitude for the following:

- Prof. C.S. van Deventer, my major promoter, for his thorough close follow-up, encouragement and keen guidance to equip me with theoretical background accompanied with his unforgettable instructive discussions of future practical applications using his ample academic experience

- Prof. M.T. Labuschagne, my co-promotor, for her commitment, invaluable intellectual advices and wholehearted supervisions during all moments of my work

- Dr. H. Maartens for creating an excellent laboratory environment, instructing me all the steps of laboratory analysis and helping me in the understanding of the results

- Ms. S. Geldenhuys for providing me excellent administrative support throughout my study

- Ms. Chrissie Miles and her quality laboratory team at ARC-Small Grain Institute, South Africa, for analyzing the grain quality of my wheat samples

- My colleagues in the breeding division and the center management at Adet agricultural research center, Ethiopia, for rendering me the necessary help to run my field experiments

- Dr. Bedada Girma and Mr. Bemnet Gashabeza, national wheat research coordinators, for their cooperation and providing me bread and durum wheat germplasm

- Prof. J.M. Carrillo at university of Madrid, Spain, for his durum wheat standards; Dr. G.Humphreys at Agriculture and Agri-food, Winnipeg, Canada, for his bread wheat standards; and Prof. M.S.Kang Louisiana State University, USA, for giving me DIALLEL-SAS analysis software; Mr. D.G. Tanner of CIMMYT Ethiopia for his encouragement and assistance

- Adet Research Center of Amhara Regional Agricultural Research Institute (ARARI) for nominating me for this opportunity and Agricultural Research Training Project (ARTP) of Ethiopian Agricultural Research Organization (EARO) for financing my scholarship

- My wife, Yehuwalashet Feleke, for her encouragement, prayers and unprecedented moral support during my study which I might not succeed with out it. I love you; appreciate your understanding, strength and courage to overcome the hard task of nursing our very young children in good and bad times while I was long time away from you

Every step in my life including completion of this undertaking is by the will of my Lord, God.

Chapter 1 Introduction

Wheat belongs to the tribe Triticeae of the grass family Poaceae and genus *Triticum* which form a polyploid series with the basic chromosome number x=7 (Moris and Sears, 1967). The bread (*Triticum aestivum* L. ssp. a*estivum*) and durum (*Triticum turgidum* L. (Thell.) *ssp. durum* (Desf.) Husn.) are the most important polyploid wheat species containing three (*AABBDD, 2n=6x=42*) and two (*AABB, 2n=4x=28*) related genomes, respectively. The main theory on the origin of the hexaploid wheat is that it evolved from the recurrent hybridizations between a cultivated tetraploid (AABB, probably *T.turgidum*) and the wild diploid (DD), *Aegilops squarrosa* (Mcfadden and Sears, 1946; Bonjean and Angus, 2001). Bread wheat is unique because its flour alone has the ability to form dough that exhibits the rheological properties required for the production of leavened bread, other foods such as flat bread, biscuits and noodles (Gainibelli et al, 2001). Durum wheat is milled into semolina and has larger kernel size, hardness and golden amber color. It is widely known to produce superior pasta products and couscous, medium-dense breads and other foods specific to certain regions (Peña et al, 2002). Wheat is grown on an area of over 200 million hectares and is now yielding almost 600 million tones annually (Marshall et al, 2001).

The genus *Triticum* in Ethiopia is represented by seven species (Vavilov, 1929) i.e., *T. dicoccum, T. durum, T .turgidum, T. polonicum, T. aestivum, T. compactum* and *T. pyrimidale.* Bread and durum are the two most widely grown important species. Bread wheat is an introduced crop, relatively unknown, in Ethiopia as opposed to durum wheat which is indigenous to the country (Hailu, 1991). The country is considered as a primary center of diversity for tetraploid wheats (Vavilov, 1931) and currently it has the greatest diversity. Ethiopia is the largest wheat producer in sub-Saharan Africa (Hailu, 1991) producing about 1.125 million tons occupying 750,000 hectares having a 55 % level of self-sufficiency (Tanner and Mwangi, 1991; Payne et al, 2001). Wheat, both durum and bread, ranks 4th in area of production (1,139,720 ha) after tef, maize and sorghum; 3rd in total production (1,571,169 tons) after tef and maize; and 2nd in productivity (1.38 tons ha^{-1}) after maize (CSA, 2001). Other than pan bread, wheat is used for making injera (pancake type bread), local bread, roasted and eaten in between meals.

The improvement of wheat since its commencement prior to the 1930's (Tessema, 1988; Hailu, 1991) has focused on improving yield and disease resistance. Since recently, the selection of varieties for higher yield preceded that of quality. The cultivar release process requires meeting agronomic superiority with relatively little emphasis on inherent quality characteristics. Currently, due to the emerging agro-industries using wheat as a raw material, industrial quality of wheat has become important (Bechere et al., 2000) and an effective strategy for wheat (bread and durum) quality improvement is required. The unsatisfactory supplies of wheat grain in terms of quality and quantity have forced some industries to import wheat from Australia and Canada (Tadesse, unpublished survey information, 2001). There is a need to strengthen the quality breeding through further exploring the genetic and environmental aspects affecting wheat quality under the wheat growing conditions of the county to meet consumer and processor demands for quality of end use products. The objectives of this study were to:

- Examine the bread making quality of widely grown commercial wheat cultivars and advanced breeding lines, identify the major contributing factors of quality under the growing conditions of northwestern Ethiopia, develop prediction models using indirect and direct measurements of quality and recommend the minimum required quality traits for possible use in the wheat breeding program of northwestern Ethiopia.
- investigate the high molecular weight, B-low molecular weight glutenins and γ-gliadin composition of Ethiopian bread and durum wheat genotypes and their associations with some physical quality traits
- study the combining ability, relative importance of additive and non-additive gene effects, genetic association and inheritance of grain and flour quality traits tested at different environments in northwestern Ethiopia

Chapter 2 Literature review

2.1 Technological quality of wheat

Wheat quality can not be expressed in terms of a single property (Finney et al., 1987). Test weight, 1000-kernel weight, and flour yield are frequently used as indicators of milling quality. Measurements of baking quality include wheat or flour protein concentration, mixing time, water absorption, loaf volume, and crumb grain and color. Several of these traits have moderate to high heritability (Braaten et al., 1962; Halloran, 1975; Loffler and Busch, 1982). The most important food use of wheat is in the manufacture of flour for bread making, biscuits, pastry products (Finney et al., 1987) and breakfast cereals. Other uses of wheat include separation of its flour into starch and gluten used for industrial products, as a feed and ethanol production (Orth and Shellenbereger, 1988). Thus, the basic definition of wheat quality usually varies from one class of wheat to another. The quality of soft red or soft white wheat cultivars is its suitability for the production of cakes, cookies, and crackers. The quality of durum wheat is its suitability for semolina and macaroni production and of hard red winter and spring wheat is its specific properties that determine suitability for hard wheat milling and bread production. Thus, quality of any kind of wheat depends on several milling, chemical, baking, processing, and physical dough characteristics. End-use quality of any wheat genotype is the summation of effects of soil, climate, and seed stock on the wheat plant and the kernel components (Haunold et al.; 1962a, Finney et al., 1987).

Bread-making quality is an important but complex character of bread wheat (Pomeranz, 1988). Bread-making technology varies around the world depending on the consumer demands, the baker and technological differences (MacRitchie, 1984) and it is very difficult to formulate applicable criteria for bread-making quality. However, laboratories always resort to the use of several direct and indirect quality traits which include test weight, Zeleny or SDS-sedimentation test, Pelshenke, mixograph, farinograph, alveograph, falling number, micro baking, hardness, protein content etc. and different researchers have recommended various traits for quality evaluations.

In their study of six baking methods, Baker et al. (1971) suggested that a quality screening program should include a measure of protein content, some measure similar to farinograph

development time and some indication of the factors that affect the gassing power. Branlard et al (1991) have compared 125 European cultivars using 17 technological test giving 46 technological parameters during three years in 18 locations. The correlation studies revealed that flour yield, test weight and falling number were generally weakly correlated to the other tests; protein content affected many tests; Zeleny, micro-baking, mixograph, bread-making and Pelshenke were strongly correlated (in descending order) to the other tests. The parameters giving the greatest number of significant correlations were (in descending order) modified Zeleny, alveograph strength, and mixograph height at seven min, farinograph weakening, and micro-baking. Fowler and De La Roche (1975) studied 28 different wheat kernel and flower measurements and recommended the use of kernel hardness, protein quantity and rate of dough development to provide the basic information required for estimation of the bread and/or pastry potential of a cultivar. O'Brien and Ronalds (1987) suggested that the most effective early generation selection for quality is achieved when wheats are assessed using a regime of tests that estimate grain hardness, flour protein content and some measure of potential dough strength.

Kernel hardness is very often used as an important criterion to classify wheat quality (Aamodt and Torrie, 1935; Symes, 1961; Meppelink, 1974) and related to milling and flour quality. Wheats are generally divided into two classes, hard and soft. Hard wheat requires more force to fracture kernels, maintain a larger particle size, it passes through sieves more easily, and has more damaged starch in the resultant flour. Endosperm texture is variable, and environmental factors exert modifying effects (MacRitchie, 1980; Mattern, 1988; Anjum and Walker, 1991). Protein and moisture content are important, and factors such as interaction between protein and starch, minerals, and moisture within the endosperm matrix play important roles. Bran may also affect hardness (Greenway, 1969). A vitreous (translucent or horn like) appearance is generally associated with hardness and high protein content, and opaqueness (mealyness or flouryness) with softness and low protein content (Hoseney, 1986). Hard wheats generally have high protein and tend to be vitreous, but the cause for hardness and vitreousness are different. Vitreousness is related to a lack of air spaces between granules of starch, whereas hardness relates to protein-starch bond strength and protein matrix continuity between granules. Air spaces make the opaque grain less dense and are formed during grain drying. The protein shrinks, ruptures and leaves air spaces upon drying. In vitreous kernels, the protein shrinks but remains intact (Hoseney, 1986). Low protein soft wheats are opaque or mealy, but at higher protein, the grain is or can be translucent or vitreous. This change in appearance within a cultivar does not change its kernel hardness significantly.

Test weight of normal (unshrivelled and undamaged) soft wheat grain is the combination of two factors: the density of the kernel itself and a random volume occupied by the grain (packing efficiency). It is also associated with shape of the grain (Yamazaki and Briggle, 1969). Kernel density reflects the environment in which the grain was grown and is dependent on the volume of the air in the grain as well as protein content to some extent, which also affects grain appearance. Low protein grain (mealy in appearance) has more or larger air pockets than those of relatively high protein kernels (vitreous in appearance, which may have no air pockets). Presumably, in the low protein dry wheat there is insufficient protoplasmic (protein) material to fill the interstices between starch granules in the cells. Test weight decreases with kernel deformation, especially by shriveling, and in these cases, there is an obvious increase in the ratio of bran to endosperm and concomitant yield loss.

Weight of 1000 grains can give an indication of flour yield on the basis that large, well filled, dense grain will contain a greater amount of endosperm compared with bran. However, correlations with flour yield are not particularly high. In durum wheat, however, kernel size, and therefore 1000 grain weight, is considered the best index for potential semolina yield per unit of grain (Matsuo and Dexter, 1980).

Break flour is the portion of the kernel endosperm of flour fineness obtained without crushing or reduction in the milling operation. Soft wheats fracture into significantly smaller particles than hard wheats, reflected in the greater quantity of break flour in milling (Yamazaki and Denelson, 1983). Flour yield has been shown to be a cultivar trait (Yamazaki and Andrews, 1982) and can range from 72-79 % on a products basis. Flour yield potential is better expressed when the experimental milling technique is optimized for each wheat being tested, rather than subjecting grains differing in milling response to a uniform procedure. Hard wheat of good milling quality should have normal bolting or sifting properties and therefore should be neither unusually hard nor soft. If, in addition, the wheat gives a normal yield of flour with a normal quantity of ash, almost invariably it will be suitable as good milling hard wheat. Wheat that is too hard usually requires more power and more than the normal number of break and reduction operations. The flour from such wheat usually will have relatively high ash content (Finney et al., 1987).

The amount of variation in loaf volume that can be explained by protein content varied from 1-86 % (Weegels et al., 1996). Improved bread making can be achieved by increasing protein content or by improving protein quality or both (Finney and Barmore, 1948). Protein quality is

determined by the composition of the storage proteins mainly dependent on genotype. Protein content is strongly influenced by environment and growing conditions (Graybosch et al., 1996). The glutenin content of flour could explain variation in loaf volume as well as, or better than, protein content and could be even more important than protein content in determining bread-making quality. O'Brien and Ronalds (1984) found a significant negative correlation between grain yield and flour protein content. However, grain yield was not significantly correlated with other quality measures, indicating that high yielding, good quality wheats could be obtained from the population.

Sedimentation test is the measure of protein aggregative ability and effectively provides a semi-quantitative determination of the amount of glutenin macropolymer based on the flocculation of polymeric glutenin in SDS/lactic acid solution (Axford et al., 1979; Weegels et al., 1996). There is an excellent correlation between the amount of gel protein and SDS-sedimentation volume (SDSS) and it indicates the bread-making quality of flours in a rapid and simple way. Good bread-making quality is associated with large SDS volume. Axford et al. (1979) have shown that sedimentation volume of 56 flours of 10 varieties correlated strongly with the volume of loaves from the same flours both by a long fermentation baking process and a mechanical development process. High correlations were confirmed between the amount of SDS unextractable protein and the Chopin Alveograph W and P values (Dachkevitch and Autran, 1989), and SDSS to protein content and loaf volume (de Villiers and Laubscher, 1995). Lorenzo and Kronstad (1987) found that SDSS values were highly influenced by variation in protein content of the grain as opposed to Blackman and Gill (1979) and Presten et al. (1982) who found that SDSS values were not affected by variation in the protein content in the grain. Nitrogen fertilization and locality affected SDSS volume as well as the protein content and bread volume thereby affecting the relationship between them (de Villiers and Laubscher, 1995).

Two important bread-making properties, mixing time and mixing tolerance are objectively determined from the mixogram (Figure 1). Both the quality and quantity of proteins affect the mixing (physical dough) properties of bread wheat flours (Finney and Shogren, 1972). Mixograph mixing time is more highly correlated with experimental bake mixing time than is Farinograph mixing time (Miller et al., 1956; Shellenberger et al., 1970). In general, the longer the mixing time is, the better the mixing tolerance. A medium to medium-long mixing requirement, corresponding to the time to the peak, generally is most desirable. Dough having a short mixing time of 1.5 min almost invariably will be less stable, less elastic, and more

extensible than dough having a longer mixing time of 2.5 to 3 min. In general, as mixing time increases, dough extensibility decreases and dough stability and elasticity increases (Grass et al, 2001).

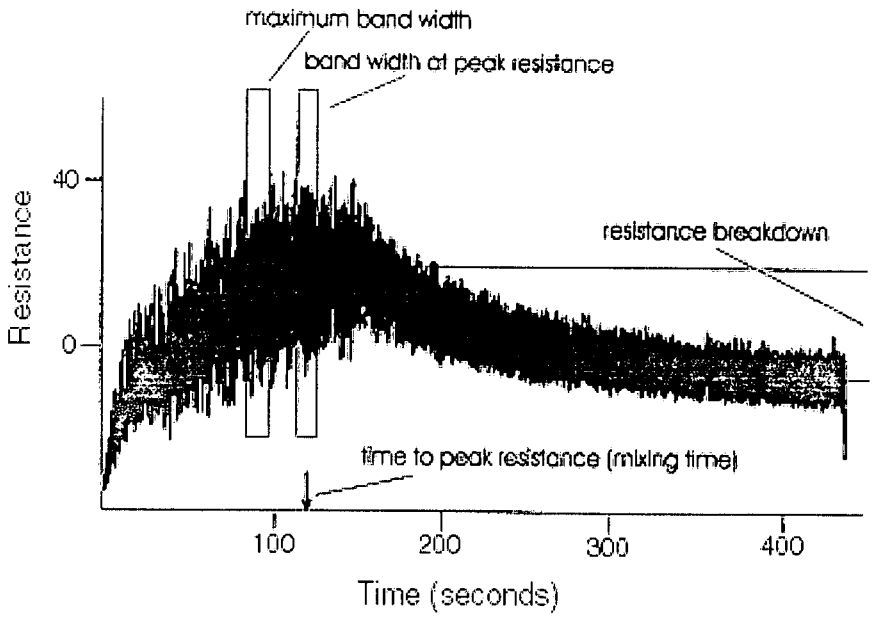


Figure 1. Mixograph measurements. The x-axis tracks the time since the start of mixing and the y-axis measures the instantaneous levels of power required to mix the dough. The width of the trace reflects the repeated stretching and rupture of the dough as it is mixed (Source: Grasa et al., 2001)

As protein content increases within a cultivar, water absorption increases (Finney, 1945). The water absorption at a given protein level varies between cultivars indicating it is a function of quantity and quality of wheat flour proteins. Correlations between baking data and mixograph characteristics were substantiated (Dong et al., 1992; Khatkar et al., 1996), and proved to be a powerful tool to investigate indices of bread making quality (Martinant et al., 1998), and mixogrpph characterstics are used as an official physical test in many countries. Dong et al. (1992) found significant correlations between protein concentration and both loaf volume and absorption and between mixing tolerance and crumb grain score. No association was found between total protein content and mixing properties. High molecular weight glutenin subunits (HMW-GS) 5+10 had the most consistently positive effect on most of the quality measurements. They concluded that using biochemical methods to identify wheat genotypes with specific HMW glutenin and gliadin composition in parental and early generation selection, but phenotypic quality traits must be considered as well.

Alveograph is widely used to determine the bread-making quality of wheat. It measures dough resistance, elasticity and protein strength of bread wheats. Yamamotto et al (1996) after investigation of four classes of soft wheats recommended the use of the alveograph and mixograph as useful tools for evaluation of soft wheat quality for cake and cookie baking. Farinograph is the most widely used physical dough testing instrument. It may be used to estimate absorption, optimum mixing time, and mixing tolerance, provided the necessary correlations have been established (Shellenberger et al., 1970).

Loaf volume is a function of both the quantity and quality of flour proteins (Finney et al., 1987). A flour of good quality for bread-making should have a high water absorption, a medium-long mixing requirement, a small to medium oxidation requirement, satisfactory mixing tolerance and dough handling properties, and good loaf volume potential (considering protein content) (Finney et al., 1987). Also, it should yield a loaf that has good internal crumb grain and color. Loaf volume at the 13 % protein level increases as mixing time increases up to about 3 min. Beyond 3 min, loaf volume is approximately constant with increasing mixing time. The lowest loaf volumes for mixing time greater than 3 min are considered to be barely satisfactory. Thus, mixing time or requirement obtained from the mixogram is a reliable index of loaf volume potential and protein quality, when selecting cultivars that have mixing requirements of about 3 min or greater (Finney et al., 1985).

The end-use quality of any wheat genotype can vary tremendously depending on the geographical production environment, soil N availability, temperature during grain filling, distribution of rainfall, and late season frosts (Haunold et al., 1962b; Faridi and Finley, 1989; Randam and Moll, 1990; Johnson and Marten, 1987; Graybosch et al., 1996). Peterson et al (1992) found that genotype, environment, and interaction effects significantly influence the variation in all quality parameters and variances of quality characteristics associated with environmental effects were generally larger than those for genetic factors. It is known that grain yield and protein concentration often are negatively correlated in cereal crops, especially when soil nitrogen is limiting (Haunold et al., 1962b). Cox et al. (1989) after examining the changes in quality of releases from 1874-1988 concluded that deterioration in quality may be caused by nongenetic factors such as changes in the environment, milling practices, commercial baking methods and formulations, or some combinations of these factors. In a genotype by environment study of soft wheats, Basset et al. (1989) found significant effects of cultivar and environment in flour yield, percent flour protein, hardness and sedimentation, alkaline water retention capacity (AWRC) and cookie diameter. Genotypes by environment

interactions were small, but significant. Among the variance components, years contributed most to total variance for protein, sedimentation and AWRC. The year by site component was greatest for flour yield, cookie diameter and hardness. Relatively large cultivar by environment components for cookie diameter, hardness, and AWRC required their evaluation across multiple site years.

2.2 Polymorphism and functional role of storage proteins

The unique properties of the wheat grain reside primarily in the gluten forming storage proteins of its endosperm which gives its viscoelastic properties. The essence of elasticity is that, following extension, a restoring force exists which tends to return the material to its original dimensions. For most traditional uses, wheat quality derives mainly from two interrelated characteristics: grain hardness and "protein quality". Quality is determined by the molecular structure of the storage proteins of wheat, that in turn, controls the interactions of the proteins during the bread making process (Payne, 1987; Bushuk, 1998; Gainibelli et al., 2001).

Based on their sequential extraction and differential solubility, Osborne (1907) classified wheat proteins into four different groups, albumins (soluble in water and dilute buffers), globulins (not soluble in water but soluble in saline solutions), prolamins (which are soluble in 70–90 % ethanol), and glutelins (which are soluble in dilute acid or alkali). Later on, Chen and Bushuk (1970) added a fifth fraction by dividing glutenin into two fractions: one soluble in dilute acetic acid (0.05*M*) and other insoluble in this solvent. However, each of these fractions is a complex mixture of different polypeptides and that these polypeptides overlap in their solubilities particularly the gliadin and glutenin proteins.

After the reduction of disulfide bonds, all gluten proteins are soluble in 70 % ethanol or other alcohol such as n-propanol as individual polypeptide chains (Kreis et al., 1985). They have thus been classified as prolamins due to the existence of close similarity in structure between low molecular weight glutenin subunits (LMW-GS) and gliadins (Shewry et al., 1986). All gluten proteins are high in proline and glutamine contents, the name prolamins being derived from the combined names of these amino acids. Within this group, further differences between them are based on biochemical characteristics: sulfur-rich, sulfur-poor, and HMW prolamins (Figure 2). While gliadins are single polypeptide chains (monomeric

proteins), the glutenins are multi-chained structures of polypeptides that are held together by disulfide bonds. The very high molecular weight of these polymeric structures is responsible for their partial insolubility and for their distinct contribution to functionality compared with that of the gliadins.

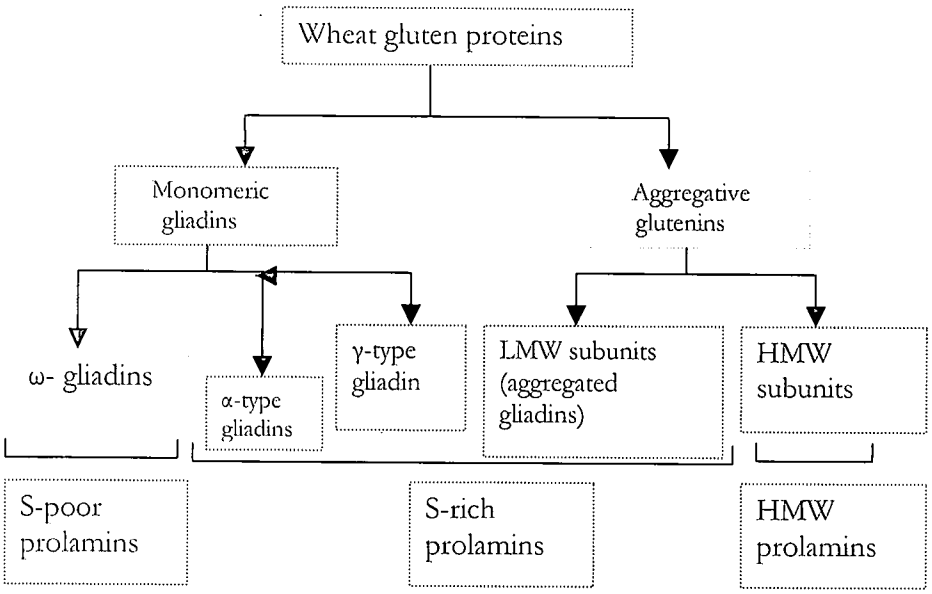


Figure 2. Comparison of the traditional (functional) and new (molecular) classification of gluten proteins (Shewry et al., 1986)

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) is the procedure most widely used to separate the reduced subunits of glutenin. In SDS-PAGE, SDS masks protein charge, and thus separation depends only on size. This shows two groups of bands, which have been called the high molecular weight (HMW, 80,000-130,000 *Da*) and low molecular weight (LMW, 10,000-70,000 *Da*) subunits (Bietz and Wall, 1972; Payne et al., 1980; Jackson et al., 1983). True estimates calculated from derived amino acid sequences indicate lower molecular weights for HMW-GS (Anderson et al., 1989; Anderson and Green, 1989).

According to the scheme of Payne et al. (1979), HMW-GS were termed A subunits, LMW-GS were further broken down into two groups, B-subunits (slower moving), and C-subunits (faster moving) distantly related to γ- and α-gliadins. Finally, the D-group, also belonging to the LMW-GS group, is highly acidic and related to ω-gliadins (Jackson et al., 1983; Masci et al., 1993; Thompson et al., 1994).

The HMW glutenin subunits of wheat protein are quantitatively minor but functionally important group of gluten proteins in the process of bread making (Tatham and Shewry, 1985; Shewry et al., 1992). They are encoded at the *Glu-1* loci on the long arm of the group 1 chromosome 1A, 1B, and 1D at *Glu-A1, Glu-B1* and *Glu-D1* loci, respectively (Payne 1987; Shewry et al., 1992; Figure 3). Each locus consists of two genes encoding a HMW x-type subunit and a HMW y-type subunit (Payne et al., 1981). Most alleles at *Glu-B1* and *Glu-D1* encode two high molecular subunits of glutenin named as x-type (lower mobility) and y-type (higher mobility) in SDS-PAGE (Rogers et al., 1991); both types of subunits are important in influencing bread making quality.

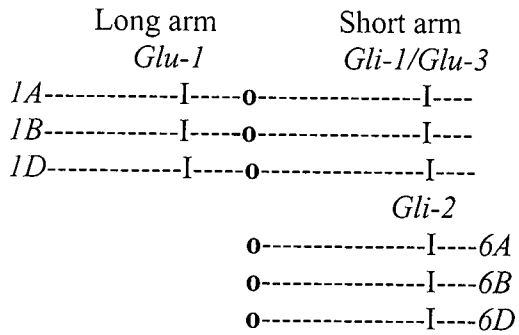


Figure 3. Chromosomal location of genes coding for gluten proteins (MacRitchie and Lafiandra, 2001)

Appreciable polymorphism was found in the number and mobility of HMW-glutenin subunits in both bread wheats (Lawrence and Shepherd, 1980; Payne et al., 1980) and pasta wheats (Waines and Payne, 1987; Branlard et al., 1989). The polymorphism provides a basis for the correlation of specific alleles with differences in bread making performance. Payne and Lawrence (1983) summarized the range of alleles at the Glu-1 loci as three allelic forms (one of which is null or silent) at the *Glu-1A,* 11 alleles (involving 14 different polypeptides) at the *Glu-1B*, and six alleles (eight different polypeptides) at the *Glu-D1* (Figure 4) which also provides the chromosomal locations of the genes. Since the publication of this catalog of alleles at *Glu-1* loci, more alleles that are novel have been identified (McIntosh et al., 1994; Gainibelli et al., 2001).

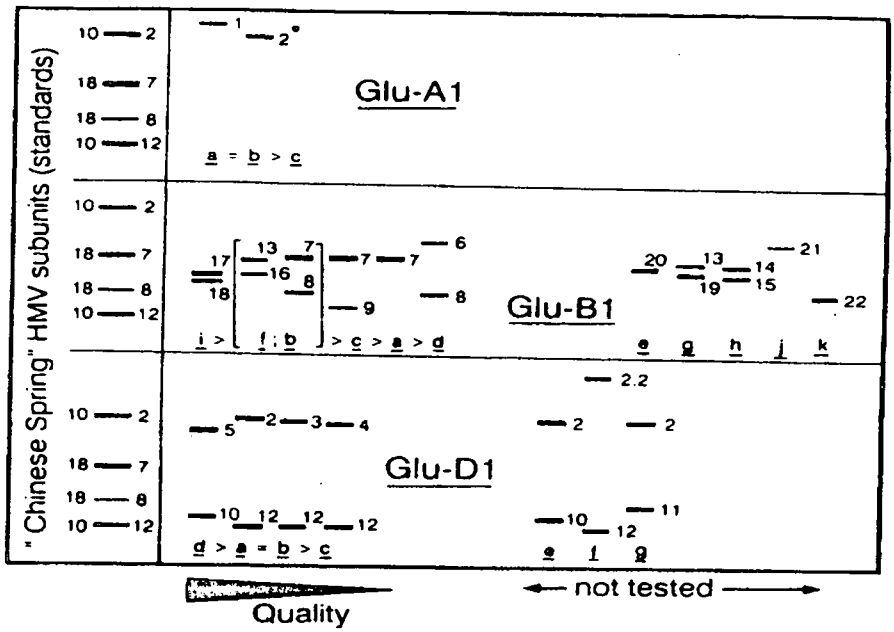


Figure 4. Allelic variation HMW glutenin subunits at three gene loci based on SDS-PAGE fractionation and relationship to bread making quality (Payne et al., 1984a). Lowercase letters refer to allele designations of Payne and Lawrence (1983) (Source: Gianibelli et al., 2001)

Bread wheat could, in theory, contain six different HMW glutenin subunits, but due to the "silencing" of some of these genes, most common wheat cultivars possess three to five HMW-GS (one to three subunits in durum wheats). Thus, all hexaploid wheats contain at least the *1Bx*, *1Dx*, and *1Dy* subunits, while some cultivars also contain *1By* and *1Ax* subunits (Gainibelli et al., 2001). It appears that the gene encoding the *1Ay* subunit is always silent. However, some bread wheats with six HMW-GS have been reported (Johansson et al., 1993; Margiotta et al., 1996) and *1Ay* subunits in A-genome diploid species (Waines and Payne, 1987) have been published. A particular feature of the HMW subunits (Shewry et al., 1989) is that they contain very large amounts of glutamine (35 mol %) and significant amounts of glycine (20 mol %) and proline (10 mol %). They are linear proteins, which consist of C and N terminal, which are largely alpha helical, separated by long domains of repeat sequences. The evidence obtained from spectroscopic results has lead to the suggestion that hydrogen bonding between the repeat regions of the HMW subunits is responsible for the elasticity of the gluten (Belton, 1999). The length and nature of the repeat region in the HMW subunits will play a role in the viscoelastic properties.

The relationship between HMW subunits and bread making quality were studied as the presence and absence of subunits (Payne et al., 1984a; Payne et al., 1987; Pogna et al., 1987; Lawrence et al., 1988) or as the quantity of one subunit is related to quality (Branlard and

Dardevet, 1985; Ng and Bushuk, 1988) and the additivity or combined role of HMW and LMW glutenin subunits in improving bread making quality (Payne et al., 1987; Gupta et al., 1989; Shewry et al., 1992). Other grain components, such as lipids and carbohydrates also affect bread making quality, possibly by interacting with the gluten proteins.

Genetic studies (Payne et al., 1981) and studies correlating HMW glutenin composition with known quality characteristics of released varieties (Payne et al., 1987) have established the presence of subunits with both positive (5+10) and negative (2+12) effects on bread making quality (Graybosch, 1992). Other allelic variant pairs had similar results: *Glu-B1* subunits 17+18 (strong) versus subunits 20x+20y (weak). These differences in dough strength were due to differences in molecular size of glutenin polymers deduced from solubility measurements (Gupta and MacRitchie, 1994). The origins of the allelic differences have not been established. However, in comparing 5+10 and 2+12, an extra cysteine residue in Dx5 was suggested as a possible explanation (Anderson and Green 1989; Kasarda, 1999). Recent advances in micro scale mixing and protein-engineering systems have proved to be valuable in elucidating structure and functional relationship in gluten proteins (Bekes et al., 1998). Branlard and Dardevet (1985) reported that the alveograph parameters W (gluten strength) and P (tenacity), and the Zeleny sedimentation value are correlated positively with subunits 7+9 and 5+10, and negatively with bands 2+12, whereas subunit 1 is correlated to W and subunits 2* and 17+18 with G (extensibility). In general, a Null at *Glu-A1* locus, subunit 4+8 encoded at *Glu-B1* and 2+12 at *Glu-D1* are negatively related to the quality parameters (Weegels et al., 1996).

Quantity of the glutenin macro-polymer content (gel protein) is statistically related to bread making quality and dough properties than individual subunits (Weegels et al., 1996). However, it is apparent that wheat flours from cultivars with HMW glutenin subunits 5+10 have larger glutenin macropolymer content. These subunits are indirectly related to good bread-making quality. Glutenin subunits or alleles could be used, however, as indicators of quality for breeding purposes, when only small amounts of material are available and fast quality prediction is necessary.

Some cultivars have better quality than expected on the basis of their HMW subunit composition, while the quality of others is unexpectedly low. In many cases, this is related to the presence of translocated 1BR/1RS translocation. However, available evidence indicates that the HMW glutamines play an important role and directly limit quality in some cultivars. Morguno et al. (1990) suggested adaptive values for particular alleles to the environmental

conditions under which the varieties were bred. The patterns of high HMW glutenin subunits amongst varieties with superior bread making quality showed few differences from from those of bread making varieties of lower quality.

A scoring system for HMW-GS has been developed (Payne et al., 1987) using major subunits encoded at the A, B, and D genome in which individual subunit pairs are graded with numbers based on bread making quality evaluations. A given cultivar can then be assigned a *Glu-1* score, which is the sum of the contributions of each of the three HMW-GS loci. Many breeding programs have characterized the HMW-GS composition of their breeding and released lines/cultivars (Lawrence, 1986; Payne, 1987; Rogers et al., 1989; Morguno et al., 1990; Lukow, 1991; Lookhart et al., 1993; Igrejas et al., 1999; Nakamura, 2001) in relation to end-use quality and used it as a screening test to ensure that good bread making alleles (1, 2*, 7+9, 7+8, 5+10) are incorporated into new cultivars (Lukow, 1991).

The HMW-GS score has more influence in some sets of wheats than in others (MacRitchie et al., 1990) and lines with poor banding combinations (2+12) produced a relatively high volume (Lorenzo and Kronstad; 1987, Bedõ et al, 1995). This is likely to be due to the complex interaction of factors that define wheat quality. These factors, in which HMW-GS have a major role, also include LMW-GS, gliadins, and abiotic stresses. Nevertheless, reference to HMW-GS composition has proved valuable in the segregation of lines in the process of breeding for specific quality targets (Weegels et al., 1996; Cornish et al., 1999).

Studies indicated that variation in HMW subunits of genotypes accounts 20-60 % of the variation in bread making quality (Payne et al., 1987; Lukow et al., 1989; Rogers et al., 1989; Kolster et al., 1991). When protein content was below 9.2 %, no effect of allelic variation at the *Glu-D1* locus was present (Kolster et al., 1991). Epistatic effects between *Glu-1* loci also contributed to the variation in loaf volume of the lines; for example, the effect of allelic variation at *Glu-A1* and *Glu-B1* depend on the allele present at *Glu*-D1. Branlard (1987) who studied genotypes with a protein content varying from 10 to 19 % found effects of HMW alleles on the bread making quality between 10 % and 15 % protein and no effects when the protein exceeds 15 %. Therefore, the variation in HMW allele composition depends on the protein content of the flour used in the baking test.

Lorenzo and Kronstad (1987) evaluated SDSS and SDS-PAGE to identify the protein quality factors from different sources of high protein bread wheat selections. They concluded that

electrophoresis analysis (SDS-PAGE) of HMW-GS was a reliable indicator of loaf volume at specific protein levels. Bands 5+10 contributed from the D genome, with either bands 1 or 2* from the A genome and bands 7 and 8 or 17 and 18 coded by the B genome were correlated with high volume. HMW glutenin banding patterns were independent of environmental factors and could help in identifying good bread making quality in low protein environments but this technique might not be as reliable in continental climates where loaf volume can be a function of high protein content. The values of SDSS discriminated between wheat lines giving high or low loaf volume, but SDSS values were found to be dependent upon variation in protein content for the experimental material used.

Brunori et al. (1989) studied relationships of dough strength and mixing stability in relation to flour protein content, glutenin/gliadin ratio, and high molecular weight subunits in bread wheat progenies. They concluded that genotype selection based on HMW subunits of glutenins would have a beneficial effect on dough strength (W). High W could be associated with a high glutenin/gliadin ratio as well as with the presence of specific HMW subunits of glutenin. They also suggested a reasonable breeding objective could be the development of wheat varieties with medium expression of gluten quality sufficient for bread making purposes. Varieties of this type may be expected to maintain a practically constant bread making quality through the years, especially in respect to the expression of a balanced P/L ratio.

Kolster et al. (1991) studied 226 lines, which were not selected for quality, and found that 20 % of the total variation in loaf volume was accounted for by the variation in HMW glutenin subunits. Most important was the allelic variation at the *Glu-D1* locus, the glutenin allele encoding the subunit 5+10 was superior to its allelic counterpart, encoding 2+12. The difference in average of loaf volume between groups of lines containing 5+10 or 2+12 was negatively correlated with protein content of the flours. When the protein was below 9.2 %, no effect of allelic variation at the *Glu-D1* locus was present.

Campbell et al. (1987) used a wide range of materials from very different breeding programs chosen on the basis of agronomic characters rather than grain quality, to overcome previous reports by others for small scale quality tests and for different sets of wheats. In their biochemical study of HMW, using Payne and Lawrence's (1983) classification, consistent prominence of *Glu* 5+10 (producing strong wheat) and *Glu* 2+12 (associate with poor quality) was found consistent with previous studies (Payne et al., 1981; Payne and Lawrence, 1983). These proteins are associated with the D genome, the one that distinguishes bread wheat from

durums. This probably explains why HMW glutenin subunits have not been associated with dough properties in durum wheats (Du Cross, 1987). In the same study, they reported the strong association of gliadins (58, 59, 60 67) with dough resistance. The associations have a strong genetic basis and are thus valuable in breeding.

It was suggested (Payne et al., 1981), that bringing together those subunits which correlate strongly with bread-making quality by conventional breeding using the SDS-sedimentation test as a primary screen and SDS-PAGE as a secondary screen to test the desired subunits provided the effects of the good-quality subunits from each of the alleles are additive then new varieties with improved quality should result from this approach.

The determination of HMW glutenin proteins by SDS-PAGE has been evaluated as a screening test for Canadian bread wheat (Lukow, 1991). The extensive use of the back crossing method in western Canadian programs largely ensures that good bread making alleles (1, 2*, 7+9, 7+8, 5+10) are incorporated in new cultivars. Therefore, SDS-PAGE screening of early generation lines is a useful technique.

The LMW-GS (B-, C-, and D-subunits) represents about one-third of the total seed protein and ≈60 % of total glutenins (Bietz and Wall, 1973). Advances in characterization of LMW proteins are enhanced by recent SDS-PAGE (Singh et al., 1991a; Gupta and MacRitchie, 1991), RP-HPLC and capillary electrophoresis (Bean and Lookhart, 2000). Gupta and Shepherd (1990) extensively described the major LMW-GS for bread wheat (*T. aestivum* L.) based on genetic analysis and on the chromosomal location of the encoding genes and by Jackson et al. (1996). Nieto-Taladriz et al. (1997) described the allelic variation of the B-type LMW-GS in durum wheat (*T. durum*). Both systems are based on the relative electrophoretic mobility of subunits in SDS-PAGE. The LMW-GS are controlled by genes at the *Glu-A3, Glu-B3*, and *Glu-D3* loci on the short arms of chromosome 1AS, 1BS, and 1DS, respectively. On the basis of screening a collection of 222 hexaploid wheats from 32 countries, Gupta and Shepherd (1990) detected 20 different band patterns (LMW-GS blocks), six for the *Glu-A3* locus, nine for the *Glu-B3* locus, and five for the *Glu-D3* locus. Recently, two new LMW-GS with molecular weights of ≈30-31,000 Da (*Glu-D4* locus) and 32,000 Da (*Glu-D5* locus) were reported (Sreeramulu and Singh, 1997). Some cultivars do not exhibit any LMW-GS encoded by *Glu-A3*. On the other hand, there is extensive polymorphism for LMW-GS encoded by chromosome 1B. There is also evidence that some

LMW-GS are controlled by genes on group-6 chromosomes (Lew et al., 1992; Gupta and Shepherd, 1993).

Close linkage between the *Glu-3* loci encoding LMW-GS and the *Gli-1* loci has been reported (Payne et al., 1984b; Singh and Shepherd, 1984, 1988; Pogna et al., 1990). The *Gli-1* multigene loci encode γ- and ω-gliadins and some β-gliadins at the distal ends of the short arms of Chromosomes 1A, 1B, and 1D. This close linkage (estimated as 2cM between *Glu-B3* and *Gli-B1* on the short arm of chromosome 1B in both bread and durum wheat) is useful for identifying the *Glu-B3* alleles and some of the *Glu-D3* alleles in breeding programs. Because the gliadin composition can be screened more readily than specific LMW-GS, the gliadins are potentially useful as indicators of LMW-GS alleles (Singh et al., 1991b; Jackson et al., 1996). Earlier studies identified the presence of γ-gliadins 45 and 42 as reliable markers for good and poor pasta quality, respectively (Damidaux et al., 1978; Kosmolak et al., 1980). The effect of γ-gliadins on pasta quality was related to genetic linkages with LMW-GS (Payne et al., 1984b).

The allelic variation at the LMW-GS loci is associated with significant differences in dough quality in bread (Gupta et al., 1989, Gupta and MacRitche, 1994) and durum wheat (Pogna et al., 1990; Ruiz and Carrillo, 1993). LMW-GS have the ability to form large aggregates that are related to dough strength. Payne et al. (1984 b) were the first workers to associate LMW-GS with quality characters of tetraploid wheat. A preliminary study ranking LMW-GS alleles in order of quality also has been reported by Gupta et al. (1989), Cornish (1995), and Cornish et al. (1999). However, it has been suggested that the effect of these alleles on quality will be more accurately assessed if they are considered in conjunction with the HMW-GS (Gupta and MacRitche, 1994).

Gliadins are heterogeneous mixtures of single-chained polypeptides, which are, in their native state, soluble in 70 % aqueous alcohol. In accordance with their mobility in acid-PAGE (A-PAGE), they are divided into four groups: α- (fastest mobility), β-, γ-, and ω-gliadins (slowest mobility). The molecular weight range is $\approx$30,000 to 75,000 *Da*. Using one-dimensional electrophoresis, gliadins of a single wheat grain can be separated into 20-25 components (Bushuk and Zillman, 1978; Autran et al., 1979; Wrigley et al., 1982; Metakovsky et al., 1984). Two-dimensional electrophoresis allows better separation with a resolution of up to 50 components (Wrigley, 1970; Payne et al., 1982; Pogna et al., 1990). Due to extensive polymorphism, these proteins have been widely used for cultivar

identification in hexaploid and tetraploid wheats. The γ-gliadins differ from α- and β-gliadins in the amount of aspartic acid, proline, methionine, tyrosine, phenilalanine, and tryptophan (Bietz et al., 1977). The ω-gliadins differ in amino acid composition from other gliadins and do not have cysteine. The ω-gliadins are characterized by high levels of glutamine (+glutamate) (40-50 mol %), proline (20-30 mol %), and phenylalanine (7-9 mol %), which represent > 80 % of the total amino acid residues (Tatham and Shewry, 1995). All gliadins are low in the ionic amino acids (histidine, arginine, lysine, and free carboxylic groups of aspartic acid and glutamic acid). Glutamic and aspartic acids exist almost entirely as amides. Also, gliadins can be classified according to their N-terminal amino acid sequence.

Based on electrophoretic mobility, the nomenclature of gliadins uses the letters α, β, γ and ω to identify four different electrophoretic zones in acid-PAGE. An approach developed by Bushuk and Sapirstein (1991), based on previous work (Sapirstein and Bushuk, 1985), defines three arbitrary gliadin bands (40.4, 53.2, 68.6) of a reference wheat cultivar (Neepawa) as limits for the determination of the four groups: ω (< 40.4), γ (40.4-53.2), β (53.2-68.6), and α (> 68.6). Nevertheless, genetic (Payne et al., 1982) and chemical studies involving amino acid analyses and N terminal sequences (Bietz et al., 1977; Kasarda et al, 1983) suggested that the gliadins can be arranged into three major groups of α/β-, γ-, and ω-gliadins.

The genetic system of gliadin nomenclature uses two types of allelic designations. In one case, each gliadin component is identified by the chromosome on which its encoding gene is located. In the second, groups of genetically linked gliadin components are designated by an allelic block identified by the chromosomes and block letters; this is the designation currently used (Wrigley et al., 1996).

Genes coding these proteins are located on the short arms of group 1 and 6 chromosomes (Wrigley and Shepherd, 1973; Brown and Flavell, 1981). They are tightly linked genes located at three homologous loci of the group 1 chromosome: *Gli-A1, Gli-B1*, and *Gli-D1* and group 6 chromosomes: *Gli-A2, Gli-B2,* and *Gli-D2* loci. *Gli-1* genes code for all the ω- and most of the γ-gliadins while Gli-2 genes code for all the α-, most of the β-, and some of the γ-gliadins. Each cluster codes for a number of polypeptides (a block) that is inherited as a Mendelian character, and multiple allelism has been established in both *Gli-1* and *Gli-2* loci (Metakovsky et al., 1984; Metakovsky, 1991).

Contemporary work has indicated that the distribution of loci controlling ω- and γ-gliadins on group 1 chromosomes is more complex than originally supposed (Redaelli et al., 1992; Nieto-Taladriz and Carrillo, 1996; Rodriguez-Quijano and Carrillo, 1996). Some researchers have questioned the earlier conclusion of Metakovsky et al (1986), suggesting that gliadins are controlled by gene clusters at the *Gli-1, Gli-2* loci, and some other single genes separated from them. There is, thus, the concept that the genetics of gliadins should be modified to allow for the possibility that whole clusters of genes have been duplicated, as is now being revealed at the *Gli-A3* and *Gli-B3* loci (Nieto-Taladriz and Carrillo, 1996). Allelic variants of the blocks differ in the number, mobility, and intensity of their components and can be characterized through A-PAGE or even SDS-PAGE. Metakovsky and co-workers studied this group of proteins in detail. The scope of their work covered the allelic composition of gliadins from hexaploid wheat, tetraploid wheat, and diploid species related to wheat (Metakovsky et al., 1984, 1986; Metakovsky and Iakobashvili, 1990; Metakovsky and Baboev, 1992).

Gliadins are generally considered to contribute to the viscosity and extensibility of gluten. Although some authors have associated specific gliadin alleles with bread making quality, it is now accepted that these proteins may not have a direct effect on wheat quality in terms of dough strength (Gainibelli et al., 2001). This role may instead be due to the LMW-GS because of their tight genetic linkage to the gliadins. The low lysine content (0.5 mol %) of the gliadins is a major negative factor affecting the nutritional quality of the wheat proteins.

2.3 Combining ability of quality traits

Wheat quality depends on many genetic and non-genetic factors. Understanding of the inheritance of wheat quality traits, joint inheritance and their association with the environment is vital (Baker and Sutherland, 1991) to improve wheat quality. Combining ability analysis of diallel crosses is useful to understand the nature of gene action involved in determining quantitative traits (Griffing, 1956a,b; Baker, 1978) and identify crosses with superior performance for use in a practical breeding program. An estimation of additive and non-additive genetic components can be made from the experimental material in terms of general (*GCA*) and specific (*SCA*) combining ability variances. The *GCA* effects represent the fixable component of genetic variance (Sprague, 1966). Parents with good combining ability for a specific character may be useful in a hybridization program for the improvement of that

character. The *SCA* represents a non-fixable component of genetic variation, related to heterosis. Therefore, combining ability indicates the value of varieties as parents, identifies satisfactory hybrids and offers an indication of the mode of inheritance for the traits studied.

Quality traits are many and their inheritance is complex and shows a considerable degree of genetic variation. It has been found that progress in breeding for grain yield has been slower in the high quality bread making varieties (Shebeski, 1966; Bingham and Lupton, 1987). This may partly be attributed to the logistical difficulties of selecting for the many additional independently inherited characters of a top quality variety (Bingham et al., 1981). Edwards (1987b) and Virmani and Edwards (1983) have observed that GCA is of greater importance than SCA in wheat, although the latter appears to be more important in space plantings. Many reports suggest that GCA is the principal source of improved grain yield in hybrids, as in line varieties (Borghi et al., 1989; Morgan et al., 1989; Pickett, 1993) with small contribution from SCA. Rodriguez et al. (1967) reported high-test weights in 45 hybrids where one parent was a poor performer in this respect. Perenzin et al. (1987) and Edwards (1987a) reported mean test weights that equal or are above the means of parents. Crosses between parents of different texture have been found to produce variable results (Rodriguez et al., 1967; Edwards, 1987b). Studies on flour extraction levels in wheat crosses vary from no heterosis (Bitzer and Fu, 1972), intermediate to parents (Edwards, 1987b) and high parent or higher levels than parents.

There are several quality tests, which examine the rheological and baking properties of flour (Kent, 1983; Blackman and Payne, 1987). The SDS sedimentation test, which uses sodium dodecyl sulphate as a solution, is widely used to indicate protein quality. Brears and Bingham (1989) reported that most hybrids were intermediate in sedimentation. SDS sedimentation volume correlated with dough strength measures, and it was suggested as a useful test to select for dough strength (O'Brien et al., 1987). Quantity of protein is largely the result of environment and has been shown to be under polygenic control (Haunold et al., 1962a; Stuber et al., 1962). Protein levels in hybrids were shown to lie between parents, fall near to the lower parent and lower than the high parent. It appears that higher values can be achieved by selection of parents. Gyawali et al. (1966) found significant specific combining ability effects for grain yield, kernel weight but not for flour yield. GCA effects were significant for grain yield, kernel weight, pearling index and flour yield and they concluded interclass diversity is not necessary for heterosis. Mou et al. (1994) found negative correlation between kernel weight and protein concentration in two years and heritability estimates of 0.79 for kernel weight. Matuz et al. (1993), in crosses of Hungarian and North American winter wheat

varieties, found negative heterosis or intermediate inheritance for water absorption, development time, stability of dough, loaf volume and protein content. Borghi et al. (1987) suggested that genes for inferior dough quality acted dominant. He found crosses to be lower in dough quality than parents, sometimes approaching the lower parent. Edwards (1987a) reported that hybrids were generally intermediate in dough characteristics although there were exceptions in both directions. Most researchers have concluded that breeding for bread making quality is possible although it will be necessary to select parents carefully to achieve this (Shebeski, 1966; Rodriquez et al., 1967; Wilson, 1968; Edwards, 1987b; Borghi et al., 1987). According to review of Picket (1993), it appears that bread wheat quality is cross specific.

2.4 References

Aamodt, O.S. and Torrie, J.H., 1935. Studies on the inheritance of and the relation between kernel texture and protein content in several spring wheat crosses. *Can. J. Res. Sect.* 202.

Anderson, O. D, Green, F. C., Yip, R. E., Halford, N. G., Shewry, P. R. and Malpica-Romero, J. M., 1989. Nucleotide sequences of the two high-molecular-weight glutenin genes from the D-genome of a hexaploid bread wheat, Triticum aestivum L. cv Cheyenne. *Nucleic Acids Res.* 17:461-462.

Anderson, O. D. and Green, F. C., 1989. The characterization and comparative analysis of high-molecular-weight glutenin genes from genomes A and B of a hexaploid bread wheat. *Theor. Appl. Genet.* 77:689-700.

Anjum, F.M. and Walker, C.E., 1991. Review on the significance of starch and protein to wheat kernel hardness. *J. Sci. Food Agric.* 56:1-13.

Autran, J.-C., Lew, E. J.-L., Nimmo, C. C. and Kasarda, D. D., 1979. N terminal amino sequencing of prolamins from wheat and related species. *Nature* 282:527-529.

Axford, D.W.E., McDermott, E.E. and Redman, D.G., 1979. Note on the sodium dodocyl sulphate test of bread-making quality: comparison with Pleshenke and Zeleny tests. *Cereal Chem. 56*:582-584.

Baker, R.J, Tipples, K.H. and Campbell, A.B., 1971. Heritabilities of and correlations among quality traits in wheat. *Can. J. Plant Sci.* 51:441-448.

Baker, R.J. and Sutherland, K.A., 1991. Inheritance of kernel hardness in five spring wheat crosses. *Can. J. Plant Sci.* 71:179-181.

Baker, R.J., 1978. Issues in diallel analysis. *Crop Sci.* 18:533-536.

Bassett, L.M., Allan, R.E. and Rubenthaler, G.L., 1989. Genotype x environment interaction on soft white winter wheat quality. *Agron. J.* 81:955-960.

Bean, S. R. and Lookhart, G. L., 2000. Ultrafast capillary electrophoretic analysis of cereal storage proteins and its applications to protein characterization and cultivar differentiation. *J. Agric. Food Chem.* 48:344-353.

Bechere, E., Peña, R.J. and Mitiku, D., 2000. Quality of Ethiopian durum wheat cultivars. *In*: CIMMYT. 2000. The eleventh regional wheat workshop for eastern central and Southern Africa. Addis Ababa, Ethiopia: CIMMYT.

Bedő, Z., Kárpáti, M., Vida, G. and Kramarik-kissimon, J. and Láng., 1995. Good bread making quality wheat (*Triticum aestivum*, L.) genotypes with 2+12 subunit composition at the Glu-D1 locus. *Cereal Res. Comm.* 23(3):283-289.

Bekes, F., Tamas, L., Gras, P.W., Shewry, P.R., Anderson, O. and Appels, R., 1998. Functional domain within the glutenin subunits of wheat. *In*: Slinkard, A.E. (*Ed.).* Proc. 9th Int. Wheat Genetics Symposium, Vol. 4. Grain Quality, University of Saskatchewan: Saskatoon, Canada. *pp.* 136-138.

Belton, P. S., 1999. On the elasticity of wheat gluten. *J. Cereal Sci.* 29:103-107.

Bietz, J.A. and Wall, J.S. 1972. Wheat gluten subunits: Molecular weights determined by sodium dodecyl sulphate-polyacrylamide gel electrophoresis. *Cereal Chem.* 49:416-430.

Bietz, J. A. and Wall, J.S. 1973. Isolation and characterization of gliadin-like subunits from glutenin. *Cereal Chem.* 50:537-547.

Bietz, J.A., Huebner, F.R., Sanderson, J.E. and Wall, J.S., 1977. Wheat gliadin homology revealed through N-terminal amino acid sequence analysis. *Cereal Chem.* 54:1070-1083.

Bingham, J. and Lupton, F.G.H., 1987. Production of new varieties at integrated research approach to plant breeding. *In*: Wheat breeding, its scientific basis. (*Ed.*). Lupton, F.G.H, Pub. Chapman and Hall, London and NewYork, *pp.*487-538.

Bingham, J., Blackman, J.A. and Longbottom, W., 1981. Breeding and management of wheat varieties. *In*: Yield of cereals. Royal Agricultural society of England, National Agricultural Centre, Stoneleigh.

Bitzer, M.J. and Fu, S.H., 1972. Heterosis and combining ability in southern soft red winter wheat. *Crop Sci.*12: 35-37.

Blackman, J.A. and Gill, A.A, 1979. A comparison of some small-scale tests for bread-making quality technique for evaluation of wheat cultivars. *J. Agric. Sci.* 95:29-33.

Blackman, J.A., and Payne, P.I. 1987. Grain quality. *In:* Lupton. F.G.H (*Ed.),* Wheat breeding, its scientific basis. Chapman and Hall, London and New York. *pp.5-485.*

Bonjean, A.P. and Angus, J.W., 2001. The world wheat book. A history of wheat breeding. London: Intercept..

Borghi, B., Corbeellini, M., and Perenzin, M., 1987. Bread making quality of F_1 hybrids produced using chemical hybridizing agent. *In:* Borghi, B. (*Ed.).* Hard wheat: Agronomic, technological, biochemical and genetic aspects. Commission of the European communities, Luxembourg. *pp.*225-236.

Borghi, B., Perenzin M., and Nash, R.J., 1989. Combining ability estimates in bread wheat and performance of 100 F_1 hybrids produced using a chemical hybridizing agent. *J. Genet. Breed.* 43:11-16.

Braaten, M.O., Lebock, K.L. and Sibbitt, L.D., 1962. Intergeneration relations of physical properties of dough and carotenoid pigment content in durum wheat. *Crop Sci.* 2:277-281.

Branlard, G. and Dardevet, M, 1985. Diversity of grain protein and bread wheat quality. II. Correlation between high molecular weight subunits of glutenin and flour quality characteristics. *J. Cereal Sci.* 3:345-354.

Branlard, G., 1987. Prediction of bread wheat quality from HMW glutenins and gliadins. *In*: Lasztity, L. and Bekes, F. (*Eds.*). Glutens proteins. Proceedings of the 3rd gluten proteins workshop. Budapest, May 9-12, *pp.*604-612.

Branlard, G., Autran, J.-C. and Monneveux, P., 1989. High molecular weight glutenin subunits in durum wheat (*Triticum durum*). *Theor. Appl. Genet.* 78:353-358.

Branlard, G., Rousset, M., Loisel, W. and Autran, J.C., 1991. Comparison of 46 technological parameters used in breeding for bread wheat quality evaluation. *J. Genet. Breed.* 45:263-280.

Brears, J. and Bingham J., 1989. Exploitation of heterosis in hybrid wheat using gametocytes. *Vorträge fur pflanzenzüchtüng* 16:397-409.

Brown, J. S. and Flavell, R. B. 1981., Fractionation of wheat gliadin and glutenin subunits by two-dimensional electrophoresis and the role of group 6 and group 2 chromosomes in gliadin synthesis. *Theor. Appl. Genet.* 59:349-359.

Brunori, A., Galterio, G., Zannettino, C. and Pogna, N.E., 1989. Bread making quality indices in *Triticum aestivum* progenies. Implications in breeding for better bread wheat. *Plant Breed.* 102:222-231.

Bushuk, W., 1998. Wheat breeding for end-product use. *In:* Braun et al (Eds.), Wheat: Prospects for global improvement, Kluwer Academic publishers, Netherlands. pp. 203-211.

Bushuk, W., and Sapirstein, H.D., 1991. Modified nomenclature for gliadins. *In*: Bushuk, W. and Tkachuk, R. (*Eds.*). Gluten Proteins. Am. Assoc. Cereal Chem.: St. Paul, MN. *pp.* 454-458

Bushuk, W., and Zillman, R.R., 1978. Wheat cultivars identification by gliadin electrophoregrams. I. Apparatus method and nomenclature. *Can.J. Plant Sci.* 58:505-515.

Campbell, W.P., Wrigley, C.W., Cressey, P.J. and Slack, C.R. 1987. Statistical correlations between quality attributes and grain-protein composition for 71 hexaploid wheats used as breeding parents. *Cereal Chem.* 64(4):293-299.

Chen, C.H. and Bushuk, W., 1970. Nature of proteins in triticale and its parental species. I. Solubility characteristics and amino acid composition of endosperm proteins. *Can. J. Plant Sci.* 50:9-14.

Cornish, G.B., 1995. Wheat proteins and end-product quality. *In*: Williams, A. and Wrigley, C.W. (*Eds.*). Proc. 45th Australian Cereal Chemistry Conf., RACI: Melbourne, Australia. *pp.* 546-549Y

Cornish, G.B., Panozzo, J.F., and Wrigley, C.W., 1999. Victorian wheat protein families. *In*: O'Brien, L. Blakeney, A. B. Ross, A. S. and Wrigley, C.W. (*Eds.*). Cereals 98. Proc. 48th Australian Cereal Chemistry Conf., RACI: Melbourne, Australia. *pp.* 183-188.

Cox, T.S., Shorgen, M.D., Sears, R.G., Martin, T.G. and Bolte, L.C., 1989. Genetic improvement in milling and baking quality of hard red winter wheat cultivars, 1919 to 1988. *Crop Sci.* 29:626-631.

CSA. 2001. Report on forecast of area and production of major crops, for rural private peasant holdings, Addis Ababa, Ethiopia. Statistics bulletin 257.

Dachkevitch, T. and Autran, J.C., 1989. Prediction of bread-making quality of bread wheats in breeding programs by size-exclusion high-performance liquid chromatography. *Cereal Chem.*. 66:448-456.

Damidaux, R., Grignac, P. and Feillet, P., 1978. Mise en evidence de relations applicables en selection entre lefelectrophoregramme des gliadines et les proprietes viscoelastiques du gluten de *Triticum durum* Desf. *C R Acad. Sci. Paris* 287:701-704.

Dong, H., Sears, R.G., Cox, T.S., Hoseney, R.C., Lookhart, G.L. and Shorgen, M.D., 1992. Relationship between protein composition and Mixograph and loaf characteristics in wheat. *Cereal Chem.* 69(2):132-136

Du cros, D.L., 1987. Glutenin proteins and gluten strength in durum wheat. *J.Cereal Sci.* 5:3.

Edwards, I.B., 1987a. Baking quality of hard red winter and hard red spring wheat hybrids. *In:* Borghi, B. *(Ed.).* Hard wheat: Agronomic, technological, biochemical and genetic aspects. Commission of the European communities, Luxembourg. *pp.*215-224.

Edwards. I.B., 1987b. Breeding of hybrid varieties of wheat. *In:* hybrid seed production of selected cereals, oil and vegetable crops, FAO Plant protection and production paper 82, FAO, Rome. *pp.3-25.*

Faridi, H. and Finley, J.W., 1989. Improved wheat for baking. CRC. *Crit. Rev. Food Sci. Nutr.* 28:175-209.

Finney, K.F., 1945. Methods of estimating and the effect of variety and protein level on the baking absorption of flour. *Cereal Chem.* 22:149-158.

Finney, K., and Barmore, M., 1948. Loaf volume and protein content of hard winter and spring wheats. *Cereal Chem.* 25: 291-311.

Finney, K.F., and Shogren, M.D., 1972. A ten-gram Mixograph for determining and predicting functional properties of wheat flours. *Baker's Dig.* 46(2):32-35, 38-42, 77.

Finney, K.F., Heyne, E.G., Shorgan, M.D., Bolte, L.C. and Pomeranz, Y, 1985. Functional properties of some European wheats when grown in Europe and Kansas. *Cereal Chem..* 62:83-88.

Finney, W.T., Yamazaki, V.L., Youngs, and Rubenthaler, G.L., 1987. Quality of hard, soft and durum wheats. *In*: Heyne, E.G. (*Ed.*).Wheat and wheat improvement (2nd edition), ASA, Madison, WI. *pp.*677-748.

Fowler, D.B. and De La Roche, I.A., 1975. Wheat quality evaluation. 2. Relationship among prediction tests. *Can. J. Plant Sci.* 55:251-262.

Gainibelli, M.C., Larroque, O.R., MacRitchie, F. and Wrigley, C.W., 2001. Biochemical, genetic, and molecular characterization of wheat glutenin and its component subunits. *Cereal Chem.* 78(6):635-646.

Grasa, P.W., Anderssen, R.S., Keentok, M., Békés, F. and Appels, R., 2001. Gluten protein functionality in wheat flour processing: a review. *Aust. J. Agric. Res.* 52, 1311–1323.

Graybosch, R.A, Peterson, C.J., Shelton, R.D. and Baenziger, P.S., 1996. Genotypic and environmental modification of wheat flour protein composition in relation to end-use quality. *Crop Sci.* 36:296-300.

Graybosch, R.A., 1992. High molecular weight glutenin subunit composition of cultivars, germplasm, and parents of US red winter wheat. *Crop Sci.* 32:1151-1155.

Greenway, W.T., 1969. A wheat hardness index. *Cereal Sci. Today.* 14:4-7.

Griffing, B., 1956a. Concepts of general and specific combining ability in relation to diallel crossing systems. *Aus. J. Biol. Sci.* 9:463-493.

Griffing, B., 1956b. Generalized treatment of the use of diallel crosses in quantitative inheritance. *Heredity* 10:31-50.

Gupta, R.B. and MacRitchie. F., 1991. A rapid one-step one-dimensional SDS-PAGE procedure for analysis of subunit composition of glutenin in wheat. *J. Cereal Sci.* 14:105-109.

Gupta, R.B. and Shepherd, K.W., 1990. Two-step one-dimensional SDS-PAGE analysis of LMW subunits of glutenin. I. Variation and genetic control of the subunits in hexaploid wheats. *Theor. Appl.Genet.* 80:65-74.

Gupta, R.B. and Shepherd, K.W., 1993. Production of multiple wheat rye 1RS translocation stocks and genetic analysis of LMW subunits of glutenin and gliadins in wheat using these stocks. *Theor. Appl. Genet.* 85:719-728.

Gupta, R.B. and MacRitchie, F., 1994. Allelic variation at glutenin subunit and gliadin loci, Glu-1, Glu-3 and Gli-1 of common wheats. II. Biochemical basis of the allelic effects on dough properties. *J. Cereal Sci.* 19:19-29.

Gupta, R.B., Singh, N.K. and Shepherd, K.W., 1989. The cumulative effect of allelic variation in LMW and HMW glutenin subunits on physical dough properties in the progeny of two bread wheats. *Theor. Appl. Genet.* 77:57-64.

Gyawali, K.K., Qualset, C.O. and Yamazaki W.T., 1966. Estimates of heterosis and combining ability in winter wheat. *Crop Sci.* 8:322-324.

Hailu Gebre-Mariam, 1991. Bread wheat breeding and genetics in Ethiopia. *In:* Hailu Gebre-Mariam, Tanner, D.G., and Mengistu Hulluka, (*Eds.*). Wheat research in Ethiopia: A historical perspective. Addis Ababa. IAR/CIMMYT. *pp.* 73-93.

Halloran, G.M., 1975. Genetic analysis of grain protein percentage in wheat. *Theor. Appl. Genet.* 46:79-86.

Haunold, A., Johnson, V.A. and Schmidt, J.W., 1962a. Genetic measurements of protein in the grain of *Triticum aestivum* L. *Agron. J.* 54:1-27.

Haunold, A., Johnson, V.A. and Schmidt, J.W., 1962b. Variation in protein content of the grain in four varieties of *Triticum aestivum* L. *Agron. J.* 54:121-125.

Hoseney, R.C., 1986. Principles of Cereal Science and Technology. *American Association of Cereal Chemistry*, St Paul, MN.

Igrejas, G., Guedes-Pinto, H., Carnide, V. and Branlard, G., 1999. The high and low molecular weight glutenin subunit and ω-gliadin composition of bread and durum wheats commonly grown in Portugal. *Plant Breed.* 118:297-302.

Jackson, E.A., Holt, L.M. and Payne, P.I., 1983. Characterization of high-molecular-weight gliadin and low-molecular-weight glutenin subunits of wheat endosperm by two-dimensional electrophoresis and chromosomal localization of their controlling genes. *Theor. Appl. Genet.*66:29-37.

Jackson, E.A., Morel, M.H., Sontag-Strohm, T., Branlard, G., Metakovsky, E.V. and Redaelli, R., 1996. Proposal for combining the classification systems of alleles of Gli-1 and Glu-3 loci in bread wheat (*Triticum aestivum* L.). *J. Genet. Breed.* 50:321-336.

Johansson, E., Henriksson, P., Svensson, G. and Heneen, W. K., 1993. Detection, chromosomal location and evaluation of the functional value of a novel high Mr glutenin subunit found in Swedish wheats. *J. Cereal Sci.* 17:237-245.

Johnson, V.A. and Marten, P.J., 1987. Wheat, rye and triticale. *In*: Olson, R.A. and Frey, K.J. (*Eds.*). Nutritional quality of cereal grains: Genetic and agronomic improvement. *Agron. Monogram.* 28. ASA, Madison, WI.

Kasarda, D.D., 1999. Glutenin polymers: The *in vitro* to *in vivo* transition. *Cereal Foods World* 44:566-571.

Kasarda, D.D., Autran, J.C., Lew, E.J.L., Nimmo, C.C. and Shewry, R.P., 1983. N-terminal amino acid sequences of ω-gliadins and ω-secalins; Implications for the evolution of prolamin genes. *Biochim. Biophys. Acta* 74:138-150.

Kent, N.L., 1983. Technology of cereals. Third edition. Pergamon Press, Oxford.

Khatkar, B.S., Bell, A.E. and Schofield, J.D., 1996. A comparative study of the inter-relationship between Mixograph parameters and bread-making qualities of wheat flours and gluten. *J. Sci. Food Agric.* 72:71-85.

Kolster, P., Van Eeuwijk, F.A. and Van Gelder, W.M.J., 1991. Additive and epistatic effects of allelic variation at high molecular weight glutenin subunit loci in determining the bread-making quality of breeding lines of wheat. *Euphytica* 55:277-285.

Kosmolak, F.G., Dexter, J.E., Matsuo, R.R., Leisle, D., and Marchylo, B.A., 1980. A relationship between durum wheat quality and gliadin electrophoregrams. *Can. J. Plant Sci.* 60:427-432.

Kreis, M., Shewry, P.R., Forde, B.G., Forde, J. and Miflin, B.J., 1985. *In*: Oxford Surveys of Plant and Molecular Cell Biology. B. J. Miflin, (*Ed.*). Structure and evolution of seed storage proteins and their genes with particular references to those of wheat, barley and rye. Oxford University Press: London. *pp.* 253-317.

Lawrence, G.J., MacRitchie, F. and Wrigley, C.W., 1988. Dough and baking quality of wheat lines deficient in glutenin subunits controlled by Glu-A1, Glu-B1 and Glu-D1 loci. *J. Cereal Sci.* 7:109-112.

Lawrence, G.J., and Shepherd, K.W., 1980. Variation in glutenin protein subunits of wheat. *Aust. J. Biol. Sci.* 33:221-233.

Lawrence, G.L., 1986. The high molecular weight glutenin subunit composition of Australian wheat cultivars. *Aust. J. Agric. Res.* 37:125-133.

Lew, E.J.L., Kuzmicky, D.D. and Kasarda, D.D., 1992. Characterization of low molecular weight glutenin subunits by reversed-phase high-performance liquid chromatography, sodium dodecyl sulphate polyacrylamide gel electrophoresis, and N-terminal amino acid sequencing. *Cereal Chem.* 69:508-515.

Loffler, C.M. and Busch, R.H., 1982. Selection for grain protein, grain yield, and nitrogen partitioning efficiency in hard red spring wheat. *Crop Sci.* 22: 591-595.

Lookhart, G.L., Hagman, K. and Kasarda, D.D., 1993. High-moleclar-weght glutenin subunits of the most commonly grown wheat cultivars in the U.S. in 1984. *Plant Breed.* 110:48-62.

Lorenzo, A. and Kronstad,, W.E., 1987. Reliability of two laboratory techniques to predict bread wheat protein quality in nontraditional growing areas. *Crop Sci.* 27:247-252.

Lukow, O.M., 1991. Screening of bread wheats for milling and baking quality-a Canadian perspective. *Cereals Food World* 36(6):497-501.

Lukow, O.M., Payne, P.I. and Tkachuk, R., 1989. The HMW glutenin subunit composition of Canadian wheat cultivars and their associations with bread making quality. *J. Sci. Food Agric.* 46:451.

MacRitchie, F. and Lafiandra, D., 2001.Use of near-isogenic wheat lines to determine protein composition-functionality relationships. *Cereal Chem.* 78(5):501-506.

MacRitchie, F., 1980. Physicochemical aspects of some problems in wheat research. *In*: Pomeranz, Y. (*Ed.*). Advances in Cereal science and technology, Vol III. American Association of Cereal Technology, St Paul, MN, *pp.*271-326.

MacRitchie, F., 1984. Baking quality of wheat flour. *Adv. Food Res.* 29:201-277.

MacRitchie, F., du Cros, D.L. and Wrigley, C.W., 1990. Flour polypeptides related to wheat quality. *Adv. Cereal Sci. Technol.* 10:79-145.

Margiotta, B., Urbano, M., Colaprico, G., Johansson, E., Buonocore, F., D'Ovidio, R. and Lafiandra, D., 1996. Detection of y-type subunit at the Glu-A1 locus in some Swedish bread wheat lines. *J. Cereal Sci.* 23:203-211.

MarshallA, D.R., Langridge, P. and Appels, R., 2001. Wheat breeding in the new century-Preface. *Aust. J. Agric. Res.* i-iv.

Martinant, J.P., Nicolas, Y., Bouguennec, A., Popineau, Y., Saulnier, L. and Branlard, G., 1998. Relationship between Mixograph parameters and indices of wheat grain quality. *J. Cereal Sci.* 27:179-189.

Masci, S., Lafiandra, D., Porceddu, E., Lew, E.J.L., Tao, H.P. and Kasarda, D.D., 1993. D-glutenin subunits: N-terminal sequences and evidence for the presence of cysteine. *Cereal Chem.* 70:581-585.

Matsuo, R.R. and Dexter, J.E., 1980. Relationship between some durum wheat physical characteristics and semolina milling properties. *Can. J. Plant Sci.* 60:49-53.

Mattern, P.J., 1988. Wheat hardness: a microscopic classification of individual grains. *Cereal Chem.* 65(4):312-315.

Matuz, J., Kertész, Z. and Ács E., 1993. Inheritance of bread making quality in crosses of Hungarian and North-American winter wheats (*Triticum aestiveum* L.). *Cereal Res. Commun.*. 21(1): 39-43.

Mcfadden, E.S. and Sears, 1946. The origin of *Triticum* spelta and its free-threshing hexaploid relatives. *J. Hered.* 37:81-89.

McIntosh, R.A., Hart, G.E. and Gale, M.D., 1994. Catalogue of gene symbols for wheat (supplement). *Ann. Wheat Genet. News* 40:362-375.

Meppelink, E.K., 1974. Untersuchungen uber die methodic der Kornhartebestimmung bei Weizen. Getreide, *Mehl brot* 28: 142.

Metakovsky, E.V., 1991. Gliadin allele identification in common wheat II. Catalogue of gliadin alleles in common wheat. *J. Genet. Breed.* 45:325-344.

Metakovsky, E.V. and Baboev, S.K., 1992., Polymorphism and inheritance of gliadin polypeptides in T. monococcum L. *Theor. Appl. Genet.* 84: 971-978.

Metakovsky, E.V. and Iakobashvili, Z.A., 1990. Homology of chromosomes of *Triticum macha* Dek. et Men. and *Triticum aestivum* L. as shown with the help of genetic markers. *Genome* 33:755-757.

Metakovsky, E.V., Akhmedov, M.G. and Sozinov, A.A., 1986. Genetic analysis of gliadin-coding genes reveals gene clusters as well as single remote genes. *Theor. Appl. Genet.* 73:278-285.

Metakovsky, E.V., Novoselskaya, A.Y. and Sozinov, A.A., 1984. Genetic analysis of gliadin components in winter wheat using two-dimensional polyacrylamide gel electrophoresis. *Theor. Appl. Genet.* 69:31-37.

Miller, B.S., Hays, B. and Johnson, J.A., 1956. Correlation of Farinograph, Mixograph, sedimentation, and baking data for hard red winter wheat flour samples varying widely in quality. *Cereal Chem.* 33:277-290.

Morgan, C.L., Austin R.B., Ford M.A., Bingham J., Angus W.J. and Chowdhury, S., 1989. An evaluation of F_1 hybrid winter wheat genotypes produced using a chemical hybridizing agent. *J. Agri. Sci. Camb.* 112: 143-149.

Morguno, A.I., Rogers, W.J., Sayers, E.J. and Metakovsky, E.V., 1990. The high molecular weight glutenin subunits of Soviet grown varieties. *Euphytica* 51:41-52.

Moris, R. and Sears, E.R., 1967. The cytogenetics of wheat and its relatives. *In*: Quisenberry, K.S. and Reitz, L.P. (*Eds.*). Wheat and wheat improvement, Madison, USA. *pp.*19-87.

Mou, B., Kronstad W.E. and Saulescu, N.N., 1994. Grain filling parameters and protein content in selected winter wheat populations II. Associations. *Crop Sci.* 34:838-841.

Nakamura, H., 2001. Genetic diversity of high molecular weight glutenin subunit compositions in landraces of hexaploid wheat from Japan. *Euphytica* 120:227-234.

Ng, P.K.W. and Bushuk, W., 1988. Statistical relationships between high molecular weight subunits of glutenin and bread making quality of Canadian-grown wheats. *Cereal Chem.* 65:408-413.

Nieto-Taladriz, M.T. and Carrillo, J.M., 1996. Complexity of the Gli-A3 locus in bread wheat. *Plant Breed.* 115:192-194.

Nieto-Taladriz, M.T., Ruiz, M., Martinez, M.C., Vazquez, J.F. and Carrillo, J.M., 1997. Variation and classification of B low-molecular weight glutenin subunits alleles in durum wheat. *Theor. Appl. Genet.* 95:1155-1160.

O'Brien, L. and Ronalds J.A., 1984. Yield and quality interrelationships among random F_3 lines and their implications for wheat breeding. *Aust. J. Agric. Res.* 35:443-51.

O'Brien, L. and Ronalds J.A., 1987. Heritabilities of small scale and standard measures of wheat quality for early generation selection. *Aust. J. Agric. Res.* 38:801-808.

Orth, R.A. and Shellenbereger, J.A., 1988. Origin, production and utilization of wheat. In: Pomeranz, Y. (*Ed.*). Wheat: chemistry and technology, (vol. I). American Association of Cereal Chemists, St Paul. *pp.*1-14.

Osborne, T.B., 1907. The protein of the wheat kernel. Publication No. 84. Carnegie Institute: Washington, DC.

Payne, P.I., 1987. Genetics of wheat storage protein and the effect of allelic variation on bread making quality. *Ann. Rev. Plant Physiol.* 38:141-153.

Payne, P.I. and Lawrence, G.J., 1983. Catalogue of alleles for the complex gene loci, Glu-A1, Glu-B1, and Glu-D1 which code for the high molecular-weight subunits of glutenin in hexaploid wheat. *Cereal Res. Commun.* 11:29-35.

Payne, P.I., Corfield, K.G. and Blackman, J.A., 1979. Identification of a high-molecular-weight subunit of glutenin whose presence correlates with bread-making quality in wheats of related pedigree. *Theor. Appl.Genet.* 55:153-159.

Payne, P.I., Holt, L.M. and Law, C.N., 1981. Structural and genetic studies on the high-molecular-weight subunits of wheat glutenin. I. Allelic variation in subunits amongst varieties of wheat (*Triticum aestivum*). *Theor. Appl. Genet.* 60:229-236.

Payne, P.I., Holt, L.M., Jackson, E.A. and Law, C.N., 1984a. Wheat storage proteins: Their genetics and their potential for manipulation by plant breeding. *Philos. Trans. R. Soc. Lond.* B 304:359-371.

Payne, P.I., Holt, L.M., Lawrence, G.J. and Law, C.N., 1982. The genetics of gliadin and glutenin, the major storage proteins of the wheat endosperm. *Qual. Plant. Food Hum. Nutr.* 31:229-241.

Payne, P.I., Jackson, E.A. and Holt, L.M., 1984b. The association between γ-gliadin 45 and gluten strength in durum wheat varieties: A direct causal effect or the result of genetic linkage? *J. Cereal Sci.* 2:73-81.

Payne, P.I., Law, C.N. and Mudd, E.E., 1980. Control by homoeologous group 1 chromosomes of the high-molecular-weight subunits of glutenin, a major protein of wheat endosperm. *Theor. Appl. Genet.* 58:113-120.

Payne, P.I., Nightingale, M.A., Krattiger, A.F. and Holt, L.M. 1987. The relationship between HMW glutenin subunit composition and the bread-making quality of British-grown wheat varieties. *J. Sci. Food Agric.* 40:51-65.

Payne, T.S., Wanjama, J.K. and Girma, B., 2001. Eastern African wheat pool. *In*: Bonjean, A.P. and Angus, W.J. (*Eds.*). The wheat world book: A history of wheat breeding. London: Intercept. *pp.* 902-911.

Peña, R.J., Trethowan, R., Pfeiffer, W.H. and van Ginkel, M., 2002. Quality (end-use) improvement in wheat: compositional, genetic, and environmental factors. *In*: Basra, A.S. and Randhawa, L.S. (*Eds.*) Quality improvement in field crops. Food products press, Binghampton, USA.

Perenzin, M., Corbellini, M. and Borghi, B., 1987. Growth analysis of ten bread wheat hybrids *(T. aestivum)* produced with a chemical hybridizing Agent. *Genetica Agraria* 41: 163-172.

Peterson, C.J., Graybosch, R.J, Baenziger, P.S. and Grombacher, A.W., 1992. Genotype and environmental effects of quality characteristics of hard red winter wheat. *Crop Sci.* 32:98-103.

Picket, A.A., 1993. Hybrid wheat results and problems. Advances in plant breeding. *Supplement 15 to plant breeding.* Scientific Publishers, Berlin and Hamburg.

Pogna, N.E., Autran, J.C., Mellini, F., Lafiandra, D. and Feillet, P., 1990. Chromosome 1B-encoded gliadins and glutenin subunits in durum wheat: genetics and relationship to gluten strength. *J. Cereal Sci.* 11:15-34.

Pogna, N.E., Mellini, F. and Dal Belin Peruffo, A. 1987. Glutenin subunits of Italian common wheats of good bread making quality and comparative effects of high molecular weight glutenin subunits 2 and 5, 10 and 12 on flour quality. *In*: Borghi, B. *(Ed.)* Hard wheat: Agronomic, technological, biochemical and genetical aspects. Commission of the European Community, CEC, Rep. EUR 1172 EN, Brussels. *pp.* 53-69.

Pomeranz, Y., 1988. Composition and functionality of wheat flour components. *In*: Pomeranz, Y. (*Ed.*). Wheat: Chemistry and technology, Vol II. American Association of Cereal Chemists, St. Paul. *pp.* 219-370.

Presten, K.R., March, P.R. and Tipples, K.H., 1982. An assessment of the SDS-sedimentation test for the prediction of Canadian bread wheat quality. *Can.J. Plant Sci.* 62:545-553.

Randam, P.J. and Moll, H.J., 1990. Some effects of temperature regime during grain filling on wheat quality. *Aust. J. Agric. Res.* 41: 603-617.

Redaelli, R., Pogna, N.E., Dachkevitch, T., Cacciatori, P., Biancardi, A.M. and Metakovsky, E.V., 1992. Inheritance studies of the 1AS/1DS chromosome translocation in the bread-wheat variety Perzivan-I. *J. Genet. Breed.* 46:253-262.

Rodriguez, R., Quinones, M.A., Borlaug, N.E. and Narvaez, I., 1967. Hybrid wheats: Their development and food potential. Research Bulletin 3. International Maize and Wheat Improvement Centre, Londres, Mexico.

Rodriguez-Quijano, M. and Carrillo, J.M., 1996. Linkage map of prolamin loci Gli-D4 and Gli-D5 in hexaploid wheat. *Plant Breed.* 115:189-191.

Rogers, W.J., Payne. P.I.,, Seekings, J.A. and Sayers, E.J., 1991. Effect on breadmaking quality of x-type and y-type high molecular weight subunits of glutenins. *J. Cereal Sci.* 14:209-221.

Rogers, W.J., Payne. P.I. and Harbinger, K., 1989. The HMW glutenin subunits and gliadin composition of German grown wheat varieties and their relationship with bread making quality. *Plant Breed.* 103:89-100.

Ruiz, M. and Carrillo, J.M., 1993. Linkage relationships between prolamin genes on chromosomes 1A and 1B of durum wheat. *Theor. Appl. Genet.* 87:353-360.

Sapirstein, H.D. and Bushuk, W., 1985. Computer-aided analysis of gliadin electrophoregrams. I. Improvement of precision of relative mobility determination by using three reference band standardization. *Cereal Chem.* 62:372-377.

Shebeski, L.H., 1966. Quality and yield studies in hybrid wheat (*Triticum aestivum* L.). *Can. J. Genet. Cyto.* 8:375-386.

Shellenberger, J.A., Pomaranz, Y., Finney, K.F. and Ward, A.B., 1970. Chemical, rheological and bread-making characteristics of selected varieties of various wheat classes. *Baker's Dig.* 44(5):27-31, 34-35.

Shewry, P.R., Halford, N.G. and Tatham, A.S., 1989. The high molecular weight subunits of wheat, barley and rye: Genetics, molecular biology, chemistry and role in wheat gluten structure and functionality. *In*: Miflin, J. (*Ed.*) Oxford Surveys of Plant and Molecular Cell Biology, Vol. 6.B. Oxford University Press: London. *pp.*163-219.

Shewry, P.R., Halford, N.G. and Tatham, A.S., 1992.High molecular weight subunits of wheat glutenin. *J. Cereal Sci.* 15:105-120.

Shewry, P.R., Tatham, A.S., Forde, J., Kreis, M. and Miflin, B.J., 1986. The classification and nomenclature of wheat gluten proteins: A reassessment. *J. Cereal Sci.* 4:97-106.

Singh, N.K. and Shepherd, K.W., 1984. A new approach to studying the variation and genetic control of disulphide-linked endosperm proteins in wheat and rye. *In:* Graveland, A. and Moonen, J.H.E. (*Eds.*). Proc. 2nd International Workshop of Gluten Proteins, TNO:Wageningen, The Netherlands. *pp.* 129-136.

Singh, N.K. and Shepherd, K.W., 1988. Linkage mapping of the genes controlling endosperm proteins in wheat. 1. Genes on the short arms of group 1 chromosomes. *Theor. Appl. Genet.* 66:628-641.

Singh, N.K., Shepherd, K.W. and Cornish, G.B., 1991a. A simplified SDS-PAGE procedure for separating LMW subunits of glutenin. *J.Cereal Sci.* 14:203-208.

Singh, N.K., Shepherd, K.W., Gupta, R.B., Moss, H.J. and MacRitchie, F., 1991b. Proportion of glutenin in the total flour protein as a measure of dough strength. *In*: W. Bushuk and R. Tkachuk (*Eds.*). 1990. Gluten Proteins., Am. Assoc. Cereal Chem.: St. Paul, MN. *pp.* 129-144.

Sprague, G.F., 1966. Quantitative genetics in plant improvement. Iowa State University Press, Ames, Iowa.

Sreeramulu, G. and Singh, N.K., 1997. Genetic and biochemical characterization of novel low molecular weight glutenin subunits in wheat (*Triticum aestivum* L.). *Genome* 40:41-48.

Stuber, C.W., Johnson V.A. and Schmidt, J.W., 1962. Grain protein content and its relation ship to other plant and seed characters in the parent and progeny of a cross of *Triticum aestivum* L. *Crop sci.*2: 506-508.

Symes K.J., 1961. Classification of Australian wheat varieties based on the granularity of their wholemeal. *Aust. J. Exp. Agric. Anim. Husb.* 1:18-23.

Tanner, D.G. and Mwangi, W., 1991. Current issues in wheat research and production in Eastern, Central and Southern Africa. *In:* Tanner, D.G, and Mwangi, (*Eds.*). Constraints and achievements. Seventh regional wheat workshop for Eastern, Central and Southern Africa, Nakuru, Kenya: CIMMYT. *pp.*17-36.

Tatham, A.S. and Shewry, P.R., 1985. The conformation of wheat gluten proteins. The secondary structures and thermal stabilities of alpha-, beta-, gamma- and omega- gliadins. *J. Cereal Sci.* 3:103-113.

Tatham, A.S. and Shewry, P.R., 1995. The S-poor prolamins of wheat, barley and rye. *J. Cereal Sci.* 22:1-16.

Tessema, T., 1988. Durum wheat breeding in Ethiopia. *In*: Van Ginkel, M. and Tanner, D.G. (*Eds.*). Fifth regional wheat workshop for Eastern, Central and Southern Africa and the Indian Ocean. Mexico, D.F.: CIMMYT. *pp.*18-22.

Thompson, S., Bishop, D.H.L., Tatham, A.S. and Shewry, P.R., 1994. Exploring disulphide bond formation in a low molecular weight subunit of glutenin using a baculovius expression system. *pp.* 345-355.

Vavilov, N.I, 1929. Wheats of Ethiopia. Bulletin of Applied Botany, Genetics and Plant Breeding 20:324-356.

Vavilov, N.I, 1931. The wheat of Abyssinia and their place in the general system of wheats, Bulletin of Applied Botany, Genetics, and Plant Breeding, Supplement 51, *p.*233.

Villiers de, O.T. and Laubscher, E.W., 1995. Use of the SDSS test to predict the protein content and bread volume of wheat cultivars. *S. Afr. J. Plant Soil.* 12(4):140-142.

Virmani, S.S. and Edwards, I.B., 1983. Current status and future prospects of breeding hybrid rice and wheat. *Advan. Agron.* 36:145-214.

Waines, J.G. and Payne, P.I., 1987. Electrophoretic analysis of the high molecular-weight glutenin subunits of Triticum monococcum, *T. erratum*, and the A genome of bread wheat (T. aestivum). *Theor. Appl. Genet.* 74:71-76.

Weegels, P.L, Hamer, R.J. and Schofield, J.D, 1996. Functional properties of wheat glutenin. *J. Cereal Sci.* 23:1-18.

Wilson.B, 1968. Problems in hybrid wheat breeding. *Euphytica* 17, Supplement 1:13-33.

Wrigley, C.W., 1970. Protein mapping by combined gel electrofocusing and electrophoresis: Application to the study of genotypic variations in wheat gliadins. *Biochem. Genet.* 4:509-516.

Wrigley, C.W. and Shepherd, K.W., 1973. Electrofocusing of grain proteins from wheat genotypes. *Ann. New York Acad. Sci.* 209:154-162.

Wrigley, C.W., Bushuk, W. and Gupta, R., 1996. Nomenclature: establishing a common gluten language. *In:* Wrigley, C.W. (*Ed.*).Gluten 96. RACI: Melbourne, Australia. *pp.* 403-407.

Wrigley, C.W., Lawrence, G.J. and Shepherd, K.W., 1982. Association of glutenin subunits with gliadin composition and grain quality in wheat. *Aust. J. Plant Physiol.* 9:15-30.

Yamamoto, H., Worthington, S.T., Hou, G. and Ng, P.K.W., 1996. Rheological properties and baking qualities of selected soft wheats grown in the United States. *Cereal Chem.* 73(2): 215-221.

Yamazaki, W.T. and Andrews, L.C., 1982. Experimental milling of soft wheat cultivars and breeding lines. *Cereal Chem.* 59:41-45.

Yamazaki, W.T. and Briggle, L.W., 1969. Components of test weight in soft wheats. *Crop Sci.* 9:457-460.

Yamazaki, W.T. and Donelson, D.H., 1983. Kernel hardness of some U.S. wheats. *Cereal Chem.* 60:344-350.

Chapter 3 Bread making quality of Ethiopian wheat cultivars and prediction using direct and indirect quality traits

3.1 Abstract

The bread making quality of Ethiopian cultivars were studied using 18 yield and quality traits at low and high protein environments. Significant variation was observed between genotypes with a broad range of milling, rheological and baking traits. Mean flour protein content, SDS-sedimentation, hardness index, mixing time and loaf volume ranged from 6.6 to 14.6 %, 28.0 to 91.7 ml, 13.2 to 62.5, 1.1 to 4.8 min and 731.3 to 1021 cm^3, respectively. Cultivars Kariega, SST 825, HAR 1868, and HAR 1685 had the highest bread volumes. These cultivars possess higher SDS-sedimentation, gluten content and mixing time. The genotypic variance ratio was consistently higher for SDS-sedimentation, mixing time and hardness index. Highly repeatable traits, in descending order, were P/L ratio, breakflour yield, SDS-sedimentation, hardness index, mixing time, loaf volume, gluten content and grain weight with values ranging from 0.63 to 0.91. Loaf volume was positively correlated with mixing time, SDS-sedimentation and gluten strength. Three different quality prediction models were constructed explaining 48 to 73 % of the variation of mixing time and loaf volume, respectively. SDS-sedimentation alone accounted for 56 % of the variation in loaf volume at the high protein environment. The variability for mixing time due to protein content alone was 37 % at the low protein environment. Both traits were common in the three models. Based on the genetic control of the traits, correlations, repeatability and predictions made, SDS-sedimentation, protein content and mixing time can be used as selection criteria where resources are limited. Hectoliter weight and grain weight also contributed to the variation of loaf volume and mixing time.

Key words: Bread wheat, breadmaking quality, prediction models

3.2 Introduction

Wheat quality refers to its suitability for the desired end product (Finney et al., 1987; Wrigley, 2002). Bread making quality is an important and complex character and its potential is determined by many direct and indirect traits including protein content, SDS-sedimentation, flour yield, mixing time, water absorption and components of the grain such as carbohydrates

and lipids and their interaction with proteins (Pomeranz, 1988; Kasarda, 1989). Genotype, growing environment and their interaction also affect many of the quality traits (Lukow and McVetty, 1991; Peterson et al., 1992; 1998). Increasing protein content or protein quality or both will help to improve breadmaking quality (Finney and Barmore, 1948). Protein content is largely affected by environment and agronomic practice whereas protein quality, the composition of storage proteins, is mainly dependent on genotype (Graybosch et al., 1996). Protein content is highly correlated to mixing tolerance, water absorption, kernel weight, SDS-sedimentation and loaf volume (Finney and Shogren, 1972; Peterson et al., 1992). SDS-sedimentation is significantly correlated with loaf volume (Greenway et al., 1996; Wikström and Bohlin, 1996) and can give the best prediction. It is also significantly correlated with protein, dough strength, extensibility, alveograph and farinograph parameters (Gröger et al., 1997). Kernel hardness determines the milling and flour yield, it is affected by genotypic rather than growing and environmental conditions (Fowler and DeRoche, 1975), and it is correlated with loaf volume. Mixograph development time is highly correlated with experimental baking time (Shellenberger et al., 1970; Dong et al., 1992; Khatkar et al., 1996) and is used for official physical dough testing in many countries (Martinant et al., 1998). Both the quantity and quality of protein affect the mixing time (Finney and Shogran, 1972). Mixing time increases at low protein levels and decreases as protein content increases. Flour water absorption increases with increasing protein content. Loaf volume, the final product, is a function of both the quantity and quality of flour proteins (Finney et al., 1987). Due to various genetic and non-genetic factors affecting wheat quality, understanding of the inheritance of quality traits, their association and influence of environment is vital (Baker and Sutherland, 1971).

Wheat in Ethiopia is a major crop occupying more than 750,000 ha producing about 1.2 million tons annually (Tanner and Mwangi, 1991) and the country is the largest wheat producer in sub-Saharan Africa (Hailu, 1991). The emerging wheat milling and baking enterprises in Ethiopia, northwestern Ethiopia in particular, depend on domestic supply of raw materials to satisfy their needs. They complained of the quality of local raw materials and lack of consistent supply to produce end-use products of acceptable quality (Tadesse, 2001, Survey supplementary to this study, unpublished). The need for improvement of grain quality in the country has become more important, due to the increasing demands from agro industries, which requires an effective strategy for quality improvement (Bechere et al., 2000; Galalcha et al., 2000). Until recently, the regional wheat breeding program focused mainly on high yield and disease resistance with limited emphasis on end use quality. The current demand forced

the consideration of breeding for both yield and quality. The objectives of these investigations were to examine the differences in bread making quality of popularly grown commercial cultivars and advanced breeding lines, quality prediction using some direct and indirect measures of quality, identification of the contribution of factors affecting quality and recommend the minimum required quality tests for the breeding program of northwestern Ethiopia.

3.3 Material and methods

Thirty cultivars/lines were sampled from commercial varieties grown by Ethiopian farmers and from advanced (fixed) breeding lines. The main criteria of selection were agronomic performance. Two South African cultivars with excellent bread making quality were included for comparison. The trials were grown at three environments: at Bainsvlei (South Africa) in 2000 and at Adet research center (Ethiopia) and Motta (Ethiopia) in 2001. RCB design with three replications was used. The plot size was 4.5 m^2 (six rows of 5 m length and 15 cm spacing between rows) at Bainsvlei and 3 m^2 (six rows of 2.5 m length and 20 cm between rows) at Adet and Motta. The recommended fertilizer rate for high yield was applied i.e., 92/46 kg N/P_2O_5 ha^{-1}. All other recommended management practices were exercised for usual wheat production. Data on yield and quality traits (Table 1) was collected and analyzed. The normality of the data was investigated for outliers. ANOVA and estimates of variance components were calculated using the restricted maximum likelihood method in SAS (1994) as follows: single environment, genotypic variance $(\sigma^2_g) = \frac{(Mg - Me)}{b}$; more environments, $\sigma^2_g = \frac{(Mg - Mi)}{bl}$; genotype by environment interaction variance $(\sigma^2_i) = \frac{(Mi - Me)}{b}$; error variance $(\sigma^2_e) = Me$; b, block; l, environment; Mg, mean square of genotype; Mi, mean square due to interaction; Me, error mean square (Singh et al., 1993). Repeatability, b_i, was calculated from the estimated components as: single environment, $b_i = \frac{\sigma^2_g}{\sigma^2_g + \sigma^2_e}$; more environments, $b_i = \frac{\sigma^2_g}{\sigma^2_g + \sigma^2_i + \sigma^2_e}$. Three sets of independent quality traits were subjected to all possible regression analysis (NCSS, 2001). The best sets out of all possible combinations were selected describing the dependent variables. Multiple regression of the selected independent quality traits was used to establish prediction equations of dependent variables using the following

simple-linear regression model: $Y = b_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \ldots + \beta_n X_n$; b_0, the intercept; β_{1-n}, the slopes of the regression plane; X_{1-n}, independent variables; Y, dependent variable. Coefficient of determination (R^2) is used to indicate the percent of variation in the dependent variable explained by selected independent variables in the model. Phenotypic correlation was calculated using Spearman's rank correlation. Genotypic correlations were computed from covariance and variance matrices as: $r_g = \frac{Cov_{xy}}{\sqrt{\sigma^2_x \sigma^2_y}}$ (Falconer and Mackay, 1996).

Table 1. Methods, units and abbreviations of measurements of quality traits

Traits	Abbreviations	Unit	Methods of measurements
Yield	GYD	ton ha^{-1}	
1000 grains weight	TGW	g	
Hectoliter weight	HLW	kg hl^{-1}	
Breakflour yield	BFLY	%	AACC 26-21A
Flour yield	FLY	%	AACC 26-21A† and Junior Quadromat mill
Flour color	FCL		Kent Johns (C76)
Flour protein content	FPC	%	AACC 39-11
Falling number	FLN	sec	AACC 56-81B
SDS-sedimentation test	SDSS	ml	AACC 56-70
Wet gluten	GLUT	%	AACC 38-12A
Vitreous kernels	VK	%	Sliced/counted
SKCS- hardness index	SKHI		AACC 53-31
SKCS-seed diameter	SKDM	mm	AACC 53-31
SKCS-seed weight	SKWT	mg	AACC 53-31
Mixograph development time	MDT	min	AACC 54-40A
Farinograph water absorption	FABS	%	AACC 54-21
Alveograph P/L ratio	P/L		AACC 54-30A
Alveograph strength	W	cm^2	AACC 54-30A
Loaf volume	LFV	cm^3	AACC 10-09

SKCS, Single Kernel Characteristics System; †, Bühler method for only blended samples; AACC, American Association of Cereal Chemists (2000)

3.4 Results and discussion

The cultivars/lines showed large variability for quality. The mean and its range for yield and quality traits at individual and combined environments are presented in Table 2 and 3. The mean FPC ranged from 10.7-14.3 at Bainsvlei, 8.7-10.6 at Adet and 6.6-8.4 at Motta. Vitreous kernels, SKHI and SDSS showed sequential increase with FPC. The highest mean value of SKHI and number of vitreous kernels were at the highest protein levels (Hoseney, 1986) and other environment effects also exert modifying effects on kernel hardness (MacRitchie, 1980; Anjum and Walker, 1991). However, the MDT had an opposite trend with FPC i.e., it

increased at lower protein levels (Johnson and Swason, 1942). The grain samples of all environments were sound with a falling number of 375-410 sec. The lowest yielding environment had high mean values for HLW, TGW and SKWT which is likely due to low stand establishment and relatively less disease pressure which produced plump and larger seeds (Table 3). The mean flour color value of most cultivars/lines was in the range for white bread (0.7-5.9). The mean loaf volume ranged from 731.3-1007.3 cm^3 at Bainsvlei, the higher protein environment, and 793-1021 cm^3 at Adet. The loaf volume of Adet was measured from blended samples of the three replications. Cultivars such as HAR 604, Kariega, SST 825 and HAR 1709 had longer MDT ranging from 2.6-4.1 min with mean protein content less than 10 % across environment. Kariega scored the highest mean loaf volume (1010.8 cm^3) with the highest SDSS value of 73.8 ml. The loaf volumes of SST 825 (981.3 cm^3), HAR 1868 (935.3 cm^3) and HAR 1685 (934.3 cm^3) were also high. These cultivars also had relatively higher GLUT, SDSS and MDT values. Cultivar HAR 604 had the highest gluten strength (W), protein content, SDSS and long MDT in the highest protein environment but with a lower gluten amount. Its high gluten strength did not secure the highest LFV (Baker and Sutherland, 1971).

Table 2. Mean of quality traits of selected cultivars/lines tested at the three environments (Bainsvlei, Motta and Adet) †

Cultivar	GYD	TGW	HLW	FLY	FPC	MDT	SDSS	VK	SKHI	SKDM	SKWT	BFLY	FCL	FABS	P/L	W	GLUT	LFV ‡
HAR 2457	4.1	37.5	75.6	65.2	9.7	2.4	45.7	63.3	44.7	2.4	34.6	22.9	2.3	66.2	1.2	28.8	37.2	787.8
HAR 2348	3.6	37.5	77.5	65.1	10.8	2.2	39.7	68.9	16.7	2.3	33.4	23.4	3.0	66.5	0.7	29.0	42.7	787.0
HAR 2807	4.0	34.9	78.3	65.9	10.0	1.9	44.1	76.4	55.6	2.2	33.4	20.3	2.0	66.2	0.7	27.6	41.9	837.5
HAR 2096	4.0	34.3	76.2	65.5	10.0	2.4	47.3	68.9	52.1	2.2	31.0	19.4	3.0	70.0	1.5	38.9	37.0	777.8
HAR 2562	3.9	36.0	78.8	66.1	10.4	2.8	46.7	78.7	58.4	2.4	35.2	21.5	3.4	64.6	0.9	31.8	36.6	812.0
ET13A$_2$	3.0	32.9	76.2	66.4	10.2	1.7	50.1	66.0	21.9	2.2	30.6	25.4	4.6	59.6	0.2	15.8	43.8	927.5
HAR 1709	3.5	32.7	77.0	66.2	9.9	2.6	56.4	71.0	47.4	2.2	30.3	25.0	5.3	66.8	0.8	47.1	44.2	877.5
HAR 1685	3.5	32.9	74.3	66.2	9.3	2.7	57.2	55.6	37.7	2.1	30.2	25.2	2.0	62.0	0.4	26.9	40.9	934.3
HAR 604	4.0	35.7	78.0	64.9	9.7	3.8	68.8	67.8	45.8	2.4	36.4	21.4	3.3	63.9	0.7	60.7	35.5	869.5
HAR 1522	3.3	29.8	78.5	64.2	9.5	4.3	53.8	68.7	56.9	2.0	25.7	22.1	2.3	62.4	0.6	41.5	33.0	872.8
HAR 1775	3.1	29.4	77.6	65.6	9.4	4.0	51.3	71.3	58.0	1.9	26.4	22.0	4.0	63.1	0.8	43.0	34.2	903.8
HAR 1868	3.9	32.4	74.4	65.8	9.0	3.4	61.4	57.8	38.2	2.1	29.5	24.3	1.3	60.9	0.4	28.3	37.3	935.3
HAR 2505	3.9	35.4	75.7	66.8	9.5	2.9	55.1	61.1	39.7	2.2	30.5	23.2	2.4	63.5	0.6	42.6	40.3	917.8
Kariega	3.9	35.1	75.9	68.4	10.0	2.9	73.8	52.7	25.9	2.3	33.3	27.0	2.7	63.2	0.3	43.1	38.8	1010.8
SST 825	4.1	33.3	76.8	66.2	9.8	4.1	62.7	68.4	51.5	2.3	30.4	21.3	2.6	65.1	0.6	56.5	36.9	981.3
Mean	3.7	34.0	76.7	65.9	9.8	2.9	54.3	66.4	43.4	2.2	31.4	23.0	2.9	64.3	0.7	37.4	38.7	882.2
Maximum	4.1	37.5	78.8	68.4	10.8	4.3	73.8	78.7	58.4	2.4	36.4	27.0	5.3	70.0	1.5	60.7	44.2	1010.8
Minimum	3.0	29.4	74.3	64.2	9.0	1.7	39.7	52.7	16.7	1.9	25.7	19.4	1.3	59.6	0.2	15.8	33.0	777.8
CV (%)	16.0	7.1	1.7	2.0	6.9	12.0	6.8	11.9	10.8	5.3	6.6	3.2	40.2	3.5	17.6	10.2	5.3	5.3
LSD (0.05)	0.5	1.9	5.5	1.0	0.5	0.3	2.8	5.8	4.5	0.1	2.0	0.8	1.7	2.9	0.2	5.3	2.7	61.3

† =30 cultivars at Bainsvlei and 15 (subset) at Adet and Motta; ‡, mean from 2 environments, one of which determined from blended samples; Yield (GYD), 1000 grains weight (TGW), Hectoliter weight (HLW), Breakflour yield (BFLY), Flour yield (FLY), Flour color (FCL), Flour protein content (FPC), Falling number (FLN), SDS-sedimentation test (SDSS), Wet gluten (GLUT), Vitreous kernels (VK), SKCS- hardness index (SKHI), SKCS-seed diameter (SKDM), SKCS-seed weight (SKWT), Mixograph development time (MDT), Farinograph water absorption (FABS), Alveograph P/L ratio (P/L), Alveograph strength (W), Loaf volume (LFV)

GYD to VK, mean of three environments; SKHI to SKWT, mean of two environments; BFLY to GLUT, mean of one environment;

Varieties grown only at Bainsvlei: HAR 1918, HAR 2258, HAR 2566, HAR 2029, HAR 2408, HAR 1896, HAR 2530, HAR 1899, HAR 1847, HAR 1920, HAR 2536, HAR 2501, HAR 2504, HAR 2192 and K6295-4A

Table 3. Mean and ranges of quality traits for selected lines tested at three environments (Bainsvlei, Adet and Motta)

	Bainsvlei		Adet		Motta		Adet x Motta		Adet x Motta x Bainsvlei	
Traits	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
GYD	7.1	5.2 - 9.1	3.4	2.6 - 4.0	2.0	1.6 - 2.4	2.7	2.1 - 3.1	3.7	3.0 - 4.1
HLW	77.0	74.0 - 79.4	75.8	72.1 - 78.3	77.6	74.4 - 80.4	76.7	73.3 - 79.3	76.7	74.3 - 78.8
TGW	38.9	33.5 - 46.5	31.2	26.0 - 35.0	33.2	28.4 - 39.0	32.2	27.2 - 36.0	34.0	29.4 - 37.5
VK	80.1	72.0 - 90.7	67.2	53.3 - 76.7	53.6	26.0 - 78.0	60.4	39.7 - 77.0	66.4	52.7 - 78.7
SKHI			47.0	13.2 - 62.5	39.7	18.5 - 59.6	43.4	16.7 - 58.3		
SKDM			2.2	1.8 - 2.3	2.3	2.0 - 2.6	2.2	1.9 - 2.4		
SKWT			30.6	24.5 - 34.9	32.2	26.9 - 38.2	31.4	25.7 - 36.3		
BFLY	21.0	17.6 - 24.5	28.8	24.3 - 34.6						
FLY	75.9	71.3 - 78.4	61.1	59.0 - 63.8	60.8	58.9 - 63.8	61.0	59.3 - 63.6	65.9	64.2 - 68.4
FCL	2.8	0.7 - 5.9								
FPC	12.6	10.7 - 14.3	9.6	8.7 - 10.6	7.3	6.6 - 8.4	8.4	7.7 - 9.2	9.8	9.0 - 10.8
SDSS	71.3	52.7 - 91.7	52.4	38.3 - 77.3	36.8	28.0 - 52.3	44.6	33.2 - 64.8	54.3	39.7 - 073.8
GLUT	38.8	31.9 - 46.8								
FLN	403.1	341 - 410	410	410	393.9	306.3-423.3			402.7	375.4- 414.3
MDT	2.1	1.1 - 3.2	2.9	1.8 - 4.8	3.8	2.1- 5.5	3.3	2.0 - 5.2	2.9	1.7 - 4.3
FABS	64.3	59.5 - 70.0								
P/L	0.7	0.2 - 1.5								
W	35.2	15.8 - 60.7								
LFV	860.8	731.3 - 1007.3	901.5	793.0 - 1021.0					882.2	777.8 – 1010.8

The analysis of variance was computed using data of Bainsvlei alone, Adet x Motta, and Adet x Motta x Bainsvlei (Table 4). The combination of environments was based on type of parameters measured and protein levels, low or high, as protein content was one of the most important parameters affecting quality associated with most rheological and end-use products which was also influenced by environment and agronomic practices (Finney and Barmore, 1948; Graybosch et al., 1993). The three environments were different in discriminating genotypes. The genotypes showed highly significant differences in all quality traits at individual and combined analysis. The genotype by environment interaction was significant for TGW, HLW, FLY, FPC and SKHI at either of the two or three environment combinations. Vitreous kernels, MDT and SDSS were significant at all combinations.

Table 4. Mean squares of quality parameters tested at three environments (Adet, Motta and Bainsvlei)

	Bainsvlei†	Adet x Motta			Adet x Motta x Bainsvlei		
	Genotype	Genotype	Env	Genotype x Env	Genotype	Env	Genotype x Env
GYD	1.8***	0.5***	44.9***	0.3	1.1***	163.1***	0.5
TGW	36.1***	42.1***	91.8***	8.1	52.3***	462.1***	11.2*
HLW	4.3***	19.2***	80.3***	1.6	18.2***	40.3***	3.6**
FLY	7.8***	9.0***	1.9***	3.6	8.4***	3240.4***	5.9***
FPC	1.5*	1.3***	126.3***	0.6*	1.8***	319.7***	0.8
MDT	1.0***	6.1***	17.6***	0.6***	6.0***	28.9***	0.7***
SDSS	362.5***	484.9***	5640.0***	42.7**	803.4***	15451.6***	44.1***
VK	83.1***	677.4***	4202.5***	136.2*	474.8***	7003.3***	204.5***
SKHI		1064.2***	1171.0***	103.0***			
SKDM		0.1***	0.3***	0.02			
SKWT		54.3***	57.7***	5.7			
BFLY	6.5***						
FCL	5.0***						
FABS	16.6***						
P/L	0.3***						
W	351.7***						
LFV	16091.3***						
GLUT	41.6***						
DF	29.0	14		14	14		28

†=30 cultivars at Bainsvlei and 15 (subset) at Adet and Motta; Env, environment
, **, * indicates significant at P<0.05, P<0.01 and P<0.001, respectively*

The variance components of genotype (σ^2_g), genotype x environment (σ^2_i) and error (σ^2_e) and their ratios were given in Table 5. All traits were significantly affected by each component of variation except σ^2_g for FPC at Bainsvlei, for FLY, FPC and GYD at Adet x Motta and for GYD, TGW, HLW and FPC at Adet x Motta x Bainsvlei. The variance due to σ^2_i was significant for SKHI at Adet x Motta and for FLY, MDT, SDSS and VK at Adet x Motta x Bainsvlei. The genotypic variance ratio was consistently higher for MDT and SDSS at single and combined environments and was also high for SKHI at two environment combinations. Similarly, variances of BFLY, P/L, W, GLUT and LFV were more genetical than other factors of variation. All other traits i.e., TGW, GYD, FPC, VK and FLY showed mixed influence of variances. The significances of genotypic variances showed that the cultivars differed with regard to their quality traits. The traits with the lowest repeatability in this study were GYD, FPC, VK and FCL (Table 5). Highly repeatable traits, in descending order, were P/L, W, BFLY, SDSS, SKHI, MDT, LFV, GLUT and SKWT ranging from 0.63-0.91. The quality traits which are less affected by environment, highly repeatable and strongly correlated with each other at each and combined environments will be vital for quality improvement (Baker and Sutherland, 1971; Branlard et al., 1991).

Table 5. Variance components and repeatability of quality parameters tested at three environments (Adet, Motta and Bainsvlei)

	Variance components					Repeatability		
	Bainsvlei	Adet x Motta		Adet x Motta x Bainsvlei				
Traits	Genotype	Genotype	Genotype x Env	Genotype	Genotype x Env	Bainsvlei	Adet x Motta	Adet : Motta Bains
GYD	0.4* (0.7)	0.032 (0.1)	0.003(0.03)	0.1 (0.2)	0.04 (0.3)	0.32(0.17)	0.09(0.07)	0.13(0
TGW	10.7*** (2.7)	5.7* (0.9)	0.6 (0.09)	4.6* (0.8)	1.8 (0.3)	0.62(0.13)	0.45(0.11)	0.38(0
HLW	1.0** (0.8)	2.9* (1.5)	-0.1 (0.0)	1.6* (0.9)	0.6 (0.4)	0.43(0.16)	0.63(0.10)	0.41(0
FLY	2.4*** (4.8)	0.9 (0.4)	0.5 (0.2)	0.3 (0.2)	1.4** (0.9)	0.83(0.07)	0.25(0.10)	0.08(0
FPC	0.2 (0.3)	0.1 (0.5)	0.1 (0.5)	0.1 (0.3)	0.1 (0.3)	0.23(0.17)	0.23(0.10)	0.16(0
MDT	0.3*** (6.0)	0.9* (5.9)	0.1* (0.9)	0.5* (4.9)	0.2** (1.7)	0.85(0.06)	0.73(0.08)	0.66(0
SDSS	118.6***(18.0)	73.7* (5.0)	9.1 (0.6)	84.4* (6.8)	10.3**(0.9)	0.94(0.03)	0.75(0.08)	0.78(0
VK	18.0* (0.6)	90.2* (1.4)	20.8 (0.4)	30.0 (0.5)	47.3**(0.9)	0.21(0.17)	0.49(0.11)	0.21(0
SKHI		160.2*(7.0)	27.0* (1.2)				0.77(0.07)	
SKDM		0.01* (1.2)	0.003 (0.8)				0.50(0.11)	
SKWT		8.1* (1.9)	0.5 (0.1)				0.63(0.10)	
BFLY	2.1*** (7.0)					0.90(0.04)		
FCL	1.1* (0.7)					0.30(0.17)		
FABS	4.1** (0.9)					0.51(0.15)		
P/L	0.08*** (4.0)					0.91(0.04)		
W	112.2***(7.4)					0.91(0.04)		
LFV	4691*** (2.3)					0.73(0.10		
GLUT	12.6*** (3.2)					0.71(0.11)		

( ), numbers in parenthesis for variance components are ratios against error component (1.0)
*( ), numbers in parenthesis for repeatability are standard errors (±SE) according to Becker (1985); *, **, *** indicates significant at P<0.05, P<0.01 and P<0.001, respectively*

Flour protein content, MDT and SDSS correlated most with other parameters. Grain yield had strong positive correlation with FLY, VK, SDSS and FPC (Table 6). It had weak negative correlation with MDT and FCL. Protein content was weakly negatively and significantly, correlated with MDT, SKDM, BFLY and LFV. At lower FPC, the MDT increased which is supported by negative correlation between them. Loaf volume was positively correlated with MDT, SDSS and W and negatively correlated with FPC, FABS and P/L. The negative correlation between LFV and FPC at Bainsvlei (Figure 8) was due to the fact that the studied cultivar sample was not originally used for simultaneous positive selection of both traits i.e., the cultivars used were not developed for quality.

Table 6. Phenotypic and genotypic correlations between direct and indirect parameters for quality traits tested at three environments (Adet, Motta and Bainsvlei)

‡‡	GYD	TGW	HLW	FLY	FPC	MDT	SDS	VK	SKHI	SKDM	SKWT	BFLY	FCL	FABS	P/L	W	LFV	GLUT
GYD		0.85	-0.31	-0.14	0.38	0	0.24	-0.01	-0.01	1.02	1.23	-0.52	-0.91	0.76	0.56	0.45	-0.08	-0.61
TGW	0.51***		-0.18	0.34	1.02	-0.67	-0.22	-0.04	-0.32	0.91	0.99	-0.11	-0.12	0.14	0.41	-0.18	-0.5	0.25
HLW	-0.11	0.24**		-1.14	0.83	0.24	-0.33	1.07	0.46	0.13	0.15	-0.32	-0.15	0.25	0.15	0.46	-0.04	-0.49
FLY	0.69***	0.44***	-0.06		0.06	-0.52	0.78	-1.14	-0.43	0.21	0.14	0.14	-0.59	0	-0.19	0.28	0.42	-0.04
FPC	0.83***	0.39***	-0.18*	0.71***		-0.81	-0.6	0.54	-0.14	0.52	0.43	0.16	0.58	0.29	-0.04	-0.14	-0.51	0.81
MDT	-0.56***	-0.42***	0.14	-0.54***	-0.64***		0.6	-0.05	0.57	-0.45	-0.62	-0.11	-0.14	0.05	-0.02	0.91	0.34	-0.7
SDS	0.77***	0.29***	-0.27***	0.67***	0.75***	-0.30***		-0.76	-0.23	0.22	0.1	0.38	-0.04	-0.42	-0.45	0.68	0.84	-0.34
VK	0.59***	0.33***	0.15	0.45***	0.73***	-0.41***	0.44***		0.62	-0.04	0.03	-0.05	0.47	0.41	0.23	0.37	-0.6	0.37
SKHI	0.14	-0.26*	0.2	-0.35***	0.30**	0.21*	0.03	0.65***		-0.31	-0.17							
SKDM	0.03	0.72***	0.29**	0.05	-0.23*	-0.09	-0.14	-0.18	-0.40*		0.9							
SKWT	0.18	0.75***	0.26*	0.11	-0.11	-0.26*	-0.11	-0.07	-0.29*	0.88**								
BFLY	-0.07	0.03	-0.24	0.21*	-0.34***	-0.1	0.21	-0.28	-0.59*	0.13	-0.06		0.41	-0.69	-0.75	-0.16	0.56	0.46
FCL	-0.43*	0.01	-0.27	-0.61***	0.52**	0.67***	-0.08	0.17				-0.05		0.05	0.04	0.17	-0.12	0.39
FABS	-0.11	0.22	-0.27	-0.38*	0.42*	0.42*	-0.53**	0.38*				-0.39*	0.32		0.93	0.28	-0.79	-0.03
P/L	0	0.22	-0.13	-0.3	0.09	-0.29	-0.57***	0.40*				-0.50**	0.07	0.76***		0.14	-0.78	-0.32
W	0.1	-0.29	0.43*	0.13	-0.29	0.86***	0.72***	-0.04				-0.21	0.03	-0.15	-0.14		0.24	-0.5
LFV[†]	-0.13	-0.49**	0.18	0.1	-0.40*	0.45*	0.85***	-0.36***	-0.47	0	-0.2	0.31**	0.03	-0.61***	-0.76***	0.45*		-0.08
GLUT	-0.2	0.17	-0.42*	-0.21	0.59***	-0.66***	-0.34	0.14				0.32	0.38**	0.33	-0.17	-0.17	-0.09	
	GYD	TGW	HLW	FLY	FPC	MDT	SDS	VK	SKHI	SKDM	SKWT	BFLY	FCL	FABS	P/L	W	LFV	

*n = 135 for GYD to VK; n = 90 for SKHI to GLUT; ‡‡; Above diagonal, genotypic correlation; Below diagonal, phenotypic correlation; GYD to VK, Correlations of three environments; SKHI to SKWT, two environments; BFLY to GLUT, one environment; *, **, *** indicates significant at P<0.05, P<0.01 and P<0.001, respectively*

End use quality of wheat depends on many direct and indirect traits with varied genetic contribution and it is desirable to select the minimum number of efficient tests describing quality (Branlard et al., 1991; Wrigley, 2002). Selection of appropriate independent variables was done by all possible regression analysis using LFV and MDT as dependent variables. The entry and stay of the predictor traits in the model during variable selection was set to P= 0.05. Three different models were constructed using multiple regressions based on the traits included, protein levels of the environments and their combination (Table 7). The data set was tested for assumption of normality and multicolinearity using the methods of omnibus (based on skewness and kurtosis) and variance inflation factor (VIF), respectively (NCSS, 2001). The first model was from data of the high protein environment (Bainsvlei); five out of 15 independent variables were selected (Table 5 and 7). In the 2nd model, six out of 11 variables were retained in the combination of low protein environments (Adet x Motta). The 3rd was a combination of all environments and four out of eight traits were chosen to describe the dependent variable. Loaf volume was the dependent variable for the first model and MDT for the rest. Mixograph development time, as direct measure of quality, significantly varied between tested genotypes, had higher genetic variance and was genetically correlated with LFV (Shellenberger et al., 1970; Dong et al., 1992). The mean values, regression coefficients, R^2 and multicolinearity test (VIF) are presented in Table 7. All the three models and regression coefficients of the selected traits in each model were significantly important to predict the dependent traits.

Model I

LFV= 849.505 - (2.975***TGW**) - (26.410***FPC**) + (4.150***SDSS**) - (61.256***P/L**) + (5.309***GLUTEN**)

Model II

MDT=-1.805 + (0.114***HLW**) - (0.587***FPC**) + (0.035***SDSS**) + (0.023***SKHI**) + (2.874***SKDM**) - (0.241***SKWT**)

Mode III

MDT = -1.428 - (0.074***TGW**) + (0.119***HLW**) - (0.344***FPC**) + (0.021***SDSS**)

Table 7. Variables, regression coefficients and R^2 values of prediction equations for quality traits

Variables	Mean (Min –Max)	Regression Coefficient	R^2 Cumulative Sequential	R^2 Alone	R^2 When omitted	Variance Inflation factor(VIF)
Model I (n=90); (Full model, R^2 = 0.73)						
Intercept		849.505				
TGW	38.9 (31.2 – 49.0)	-2.975	0.16	0.16	0.71	1.2
FPC	12.5 (10.2 - 15.5)	-26.410	0.33	0.18	0.65	1.4
SDSS	71.3 (51.0 - 93.0)	4.150	0.61	0.56	0.56	1.9
P/L	0.7 (0.18 - 1.5)	-61.256	0.68	0.34	0.69	1.4
GLUT	38.8 (28.2 - 48.1)	5.309	0.73	0.01	0.68	1.5
LFV (DV)	860.8 (655 - 1058)					
Model II (n=90); (Full model, R^2 = 0.59)						
Intercept		-1.805				
HLW	76.7 (70.8 - 81.2)	0.114	0.04	0.04	0.55	1.54
FPC	8.4 (6.3 - 11.2)	-0.587	0.25	0.25	0.26	1.48
SDSS	44.6 (24.0 - 80.0)	0.035	0.33	0.00	0.50	1.61
SKHI	43.4 (9.8 - 66.2)	0.023	0.45	0.04	0.52	1.32
SKDM	2.2 (1.7 - 2.7)	2.874	0.49	0.02	0.55	5.97
SKWT	31.4 (22.2 - 41.3)	-0.241	0.59	0.08	0.49	5.45
MDT(DV)	3.3 (1.5 - 6.0)					
Model III (n=135); (Full model, R^2 = 0.48)						
Intercept		-1.428				
TGW	33.9 (23.9 - 44.8)	-0.074	0.17	0.17	0.42	1.40
HLW	76.7 (70.8 - 81.2)	0.119	0.26	0.03	0.44	1.22
FPC	9.8 (6.3 - 15.5)	-0.344	0.43	0.37	0.28	2.48
SDS	54.3 (24.0 - 93.0)	0.021	0.48	0.11	0.43	2.31
MDT(DV)	2.9 (1.0 - 6.0)					

DV, Dependent variable; R^2, coefficient of determination

In model I, TGW and SDSS contributed, respectively, the lowest and the highest R^2. All selected independent traits (TGW, FPC, SDSS, P/L and GLUT) explained 73 % of the variations in loaf volume. Protein content, GLUT, P/L and SDSS as measures of quantity and quality of protein, and as the most important determinants of wheat quality could be used for quality selection (O'Brien and Ronalds, 1987; Bushuk, 1998). SDS-sedimentation explained 56 % of the variation (Figure 5), followed by P/L (34 %) and FPC (18 %) when each was regressed alone. Gluten content, TGW, FPC and P/L were negatively correlated with LFV and SDSS positively, all significantly.

Hectoliter weight, FPC, SDS, SKHI, SKDM and SKWT explained 59 % of MDT variation in the 2nd model. Protein content was important, expressing 25 % of the variation when regressed alone. Protein content was also limiting as it was in the lowest range as compared to the first model. As low protein content has a confounding effect on all the gluten parameters, optimum protein level was necessary for expression of gluten components and LFV (MacRitchie, 1984).

All three variables of SKCS were selected. However, SKDM had the lowest R^2 contribution and the highest VIF.

In a three environment combination of low to high protein levels, four out of eight quality traits were selected to explain MDT variation i.e., HLW, TGW, FPC and SDSS. The protein content of cultivars/lines ranged from 6.3 to 15.5 %, SDSS from 24 to 93 ml and MDT from 1 to 6 min. The model captured 49 % of the MDT variation. Protein, when regressed alone, explained 37 % of the variation of MDT (Figure 6) indicating its importance at all environments.

The residuals of actual and predicted values of LFV and MDT as percent error was generally low (Table 8) and both values were highly correlated (Figure 7) excluding few cultivars with high errors in model III. The same cultivars had the lowest MDT values in Model II and III. Cultivars having both extremes of MDT values showed larger percent errors.

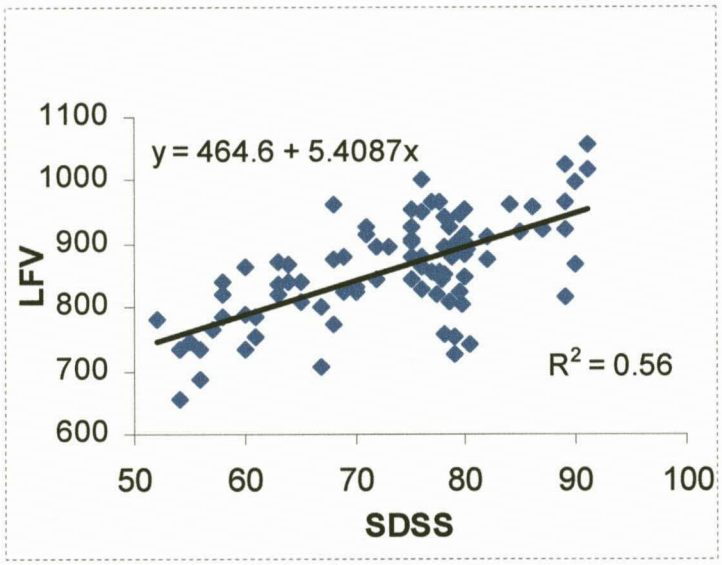


Figure 5. Relationship of SDSS alone with LFV (Model I)

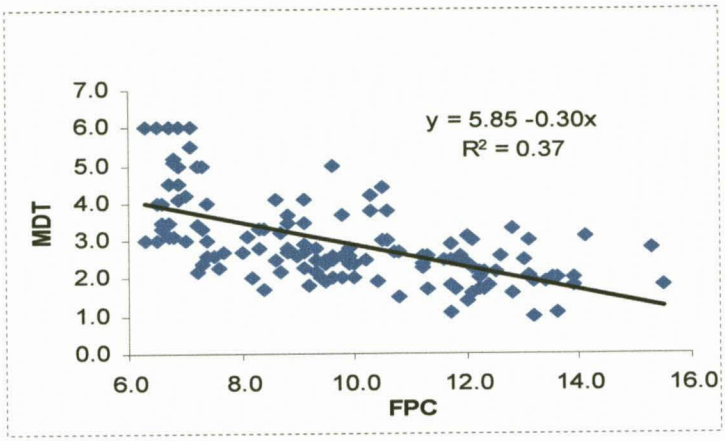


Figure 6. Relationship of FPC alone with MDT (Model III)

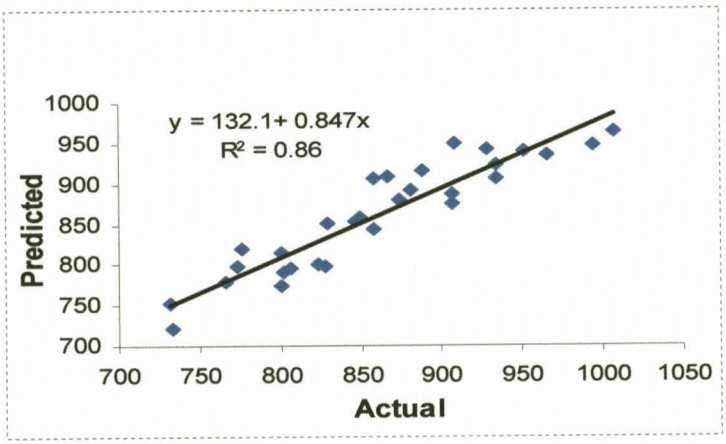


Figure 7. Correlation of measured and calculated LFV in Model I

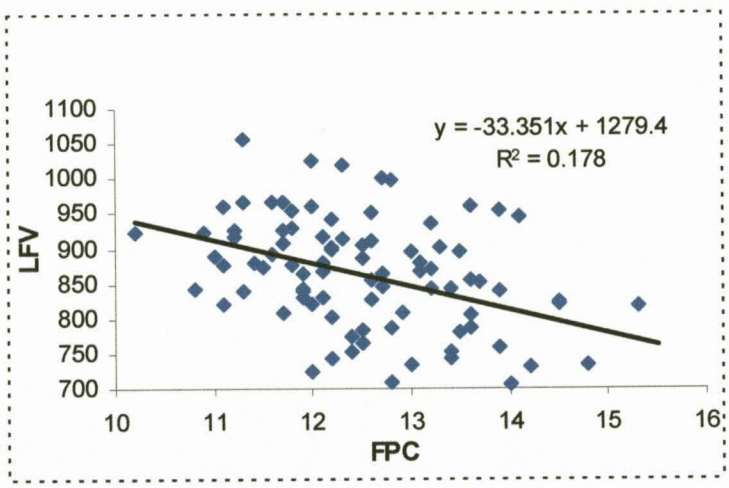


Figure 8. FPC vs LFV relationship at Bainsvlei

Table 8. Actual, predicted and percent errors of LFV and MDT using the three regression models

		Model I			Model II			Model III		
No.	NAME	LFV Actual	LFV predicted	Error (%)	MDT Actual	MDT Predicted	Error (%)	MDT Actual	MDT Predicted	Error (%)
1	HAR 2457	766.7	778.9	1.6	2.67	2.67	0.2	2.4	2.4	2.7
2	HAR 2348	731.3	752.1	2.8	2.37	1.97	16.7	2.2	2.1	3.6
3	HAR 2807	846.7	853.3	0.8	2.03	2.65	30.4	1.9	2.8	46.9
4	HAR 2096	772.7	797.2	3.1	2.67	2.98	11.7	2.4	2.7	11.1
5	HAR 2562	802.0	792.0	1.2	3.03	2.81	7.3	2.8	2.7	4
6	$ET13A_2$	933.3	906.9	2.8	2.03	2.71	33.5	1.7	2.7	60.5
7	HAR 1709	857.3	907.5	5.9	2.87	3.68	28.4	2.6	3.1	17.7
8	HAR 1685	934.3	922.0	1.3	3.13	3.53	12.5	2.7	3.0	10.4
9	HAR 604	866.7	908.1	4.8	4.25	3.89	8.5	3.8	3.3	13.6
10	HAR 1522	880.7	892.5	1.3	5.15	4.67	9.4	4.3	3.6	17.5
11	HAR 1775	907.0	886.5	2.3	4.67	4.00	14.3	4.0	3.5	13
12	HAR 1868	928.7	943.2	1.6	4.15	3.66	11.9	3.4	3.2	6.9
13	HAR 2505	906.7	875.4	3.4	3.22	3.47	8.0	2.9	2.8	3.5
14	Kariega	1007.3	963.3	4.4	2.97	3.11	4.7	2.9	3.1	8.8
15	SST-825	994.3	946.1	4.9	4.70	4.10	12.7	4.1	3.2	22.8
16	HAR 2504	965.0	934.3	3.2						
17	K6295-4A	950.3	939.3	1.2						
18	HAR 1899	907.3	948.9	4.6						
19	HAR 2192	887.7	915.4	3.1						
20	HAR 1918	806.0	794.9	1.4						
21	HAR 2258	733.7	722.6	1.5						
22	HAR 2566	823.7	801.3	2.7						
23	HAR 2029	775.3	819.6	5.7						
24	HAR 2408	828.3	799.3	3.5						
25	HAR 1896	849.3	857.6	1						
26	HAR 2530	800.3	816.2	2						
27	HAR 1847	874.0	879.6	0.6						
28	HAR 1920	857.7	844.8	1.5						
29	HAR 2536	801.0	774.3	3.3						
30	HAR 2501	828.7	850.9	2.7						
	Mean	860.8	860.8	2.7	3.3	3.3	4.3	2.9	2.9	16.2

N0 1-15 were grown at three locations (Adet, Motta and Bainsvlei); N0. 1-30, only at Bainsvlei

In this investigation, significant variability was observed among tested cultivars/lines in all traits measured at both lower and higher protein environments. Bainsvlei was the highest while Motta was the lowest protein environment The lowest protein content of the cultivars at Adet and Motta might be associated with growing conditions such as disease pressure, N-applied and associated environment effects (Johnson et al., 1979; Johnson and Marten, 1987; Graybosch et al., 1996). Cultivar 2348 had the highest protein content but the lowest SDSS, SKHI and LFV whereas HAR 604 had the highest W but lower LFV. It indicates the development of varieties with medium expression of gluten quality is sufficient for bread making purposes (Brunori et al., 1989) and with balanced P/L ratio which is necessary to help

such varieties to maintain constant bread making quality through years. Kariega had the highest mean loaf volume followed by SST 825, HAR 1868, HAR 1685 and HAR 2505. It had higher SDSS and the lowest P/L. Generally, the mean loaf volume of the cultivars at Bainsvlei (731-1007 cm^3) and at Adet (793-1021 cm^3) was high at mean protein levels of 10.7-14.3 % and 8.7-10.6 %, respectively. Cultivars HAR 604, HAR 1522 and SST 825 were hard wheats with minimum levels of proteins but with the highest MDT, SDSS, SKHI and W values. Environmental effects, genotype and their interaction were important for most quality traits (Branlard et al., 1991; Lukow and McVetty, 1991; Peterson et al., 1992; Peterson et al., 1998) but the magnitude of genotype by environment interaction was smaller for important traits. SDS-sedimentation, MDT, SKHI, W, BFLY, P/L, GLUT and LFV had higher genotypic ratios in descending order ranging from 18.0 to 2.3. The same traits had the highest repeatability (0.75 – 0.91) and simultaneous selection would be possible for those traits which are positively correlated (Baker and Sutherland, 1971; Branlard et al., 1991; Bushuk, 1998). The high repeatabilities could be also due to the presence of hard genotypes and/or the lower magnitude of genotype by environment interactions (Branlard et al., 1991). Loaf volume had positive significant correlations with SDSS, MDT, W, and BFLY and negatively correlated with P/L. Genotypic correlations also gave identical trends between these traits. SKHI had a positive significant correlation with FPC, MDT and VK i.e., the harder kernels had higher protein content (Hoseney, 1986). Both SKHI and VK as measurements of kernel hardness were highly correlated.

Prediction of LFV and MDT using the three models was very close to the measured values. Protein content, SDSS and TGW (SKWT) were common components of the three and HLW of the two prediction equations. The selected traits for prediction had positive or negative correlations with dependent variables as quality end-uses of wheat is a complex character with an interaction of various components (Pomeranz, 1988). The requirements of bread making test are small sample size, good relationship with loaf volume, independent of growing condition, in particular protein content, and simple procedure (Blackman and Gill, 1979). Axford (1978) reported that SDSS will satisfy the demands. Fowler and De La Roche (1975) and O'Brien and Ronalds (1987) recommended the use of kernel hardness, protein quantity and rate of dough development and some measure of potential dough strength to provide the basic information required for estimation of the bread and/or pastry potential of a cultivar in breeding programs.

3.5 Conclusions

Based on the results of this study, FPC and SDSS should be used as main indirect measures for wheat quality characterization. Among the direct measures studied, P/L and MDT which had the highest correlation with LFV and highest repeatability were crucial in predicting quality in these sets of materials. Although their contribution is lower, TGW and HLW were also important. In a situation where laboratory facilities are limiting in the wheat breeding program of northwestern Ethiopian, indirect measures (SKHI, SKDM, SKWT, TGW, HLW, SDSS, and FPC) combined with less expensive direct measures (MDT) will be important for quality characterization. In addition, the traits that are highly heritable and correlated with loaf volume could be evaluated at few locations or from composite samples considering the low genotype by environment interaction.

Although not deliberately selected for quality, most of the Ethiopian cultivars/lines produced a reasonably good loaf volume under medium to high protein levels and further management aspects should be recommended for wheat quality grain production. The causes of lower protein levels of cultivars/lines at two Ethiopian environments grown under recommended management for high yield needs further investigation in relation to quality traits and protein environments with further testing and characterization of wheat growing environments of the region. Breeding for good quality cultivars under medium protein environments would be possible since some cultivars scored high loaf volumes at low to medium protein levels grown at Adet research center. Protein and yield had positive genotypic and phenotypic correlations for this set of cultivars and breeding for both should be considered. It will also be important to consider separate selection for low and high protein environments. Generally, a quality breeding strategy should be developed in the region.

3.6 References

AACC (American Association of Cereal Chemists), 2000. Approved methods of the AACC. 10th edition. The association: St. Paul, MN.

Anjum, F.M. and Walker, C.E., 1991. Review on the significance of starch and protein to wheat kernel hardness. *J. Sci. Food Agric.* 56:1-13.

Axford, D.W.E., McDermott, E, E. and Redman, D.G., 1978. Note on the sodium dedocyl sulphate test of bread making quality: comparison with Pleshenke and Zeleny tests. *Cereal Chem.* 56:582-584.

Baker, R.J. and Sutherland K.A., 1971. Inheritance of kernel hardness in five spring wheat crosses. *Can. J. Plant Sci.* 71:179-181.

Bechere, B., Peña, R.J. and Mitiku, D., 2000. Quality of Ethiopian durum wheat cultivars. *In*: CIMMYT. 2000. The eleventh regional wheat workshop for Eastern Central and Southern Africa. Addis Ababa, Ethiopia: CIMMYT. *pp.* 34-44.

Becker, W.A., 1985. Manual of quantitative genetics. Fourth edition. McNaughton and Gunn, Inc. Ann Arbor, Michingan, USA.*p.*48.

Blackman, J.A. and Gill, A.A, 1979. A comparison of some small-scale tests for bread-making quality technique for evaluation of wheat cultivars. *J. Agric. Sci.* 95:29-33.

Branlard, G., Rousset, M., Loisel, W. and Autran, J.C., 1991. Comparison of 46 technological parameters used in breeding for bread wheat quality evaluation. *J. Genet & Breed.* 45:263-280.

Brunori, A., Galterio, G., Zannettino, C. and Pogna, N.E., 1989. Bread making quality indices in *Triticum aestivum* progenies. Implications in breeding for better bread wheat. *Plant Breed.* 102:222-231.

Bushuk, W., 1998. Wheat breeding for end-product use. *In:* Braun et al. (*Eds.*), Wheat: Prospects for global improvement, Kluwer Academic publishers, Netherlands. *pp.* 203-211.

Dong, H., Sears, R.G., Cox, T.S., Hoseney, R.C., Lookhart, G.L. and Shorgen, M.D., 1992. Relationship between protein composition and Mixograph and loaf characteristics in wheat. *Cereal Chem.* 69(2):132-136.

Falconer, D.S., and Mackay, T.F.C., 1996. Introduction to quantitative genetics. Fourth edition, Longman Group Ltd., England.

Finney, K.F, and Barmore, M., 1948. Loaf volume and protein content of hard winter and spring wheats. *Cereal Chem.* 25: 291-311.

Finney, K.F., and Shogren, M.D., 1972. A ten-gram Mixograph for determining and predicting functional properties of wheat flours. *Baker's Dig.* 46(2):32-35, 38-42, 77.

Finney, W.T., Yamazaki, V.L., Youngs, and Rubenthaler, G.L., 1987. Quality of hard, soft and durum wheats. *In*: Heyne, E.G. (*Ed.*). Wheat and wheat improvement (2nd edition), ASA, Madison, WI. *pp.* 677-748.

Fowler, D.B. and De La Roche, I A., 1975. Wheat quality evaluation. 2. Relationship among prediction tests. *Can. J. Plant Sci.* 55:251-262.

Galalcha, S., Debelo, D. and Bedada, G. Payne, T., Alemayehu, Z. and Yaie, B., 2000. Milling and baking quality of Ethiopian bread wheat cultivars. *In*: CIMMYT. 2000. The eleventh regional wheat workshop for Eastern, Central and Southern Africa. Addis Ababa, Ethiopia: CIMMYT. *pp.* 87-96.

Graybosch, R.A, Peterson, C.J., Moore, K.J., Stearns, M. and Grant, D.L., 1993. Comparative effects of wheat flour protein, lipid and pentosan composition in relation to baking and milling quality. *Cereal Chem.* 70(1):95-101.

Graybosch, R.A, Peterson, C.J., Shelton. R.D. and Baenziger, P.S., 1996. Genotype and environmental modification of wheat flour protein composition in relation to end-use quality. *Crop Sci.* 36:296-300.

Greenway, W.T, Hurst, N.S., Neustadt, M.H. and Zeleney, L., 1996. Micro sedimentation test for wheat. *Cereal Sci. Today* 11:179-199.

Gröger, S., Oberforster, M., Wertenker, M., Grausgruber, H. and Lelley, T., 1997. HMW glutenin subunit composition and bread making quality of Australian grown wheats. *Cereal Res. Comm.* 25(4):955-962.

Hailu Gebre-Mariam, 1991. Bread wheat breeding and genetics in Ethiopia. *In:* Hailu Gebre-Mariam, Tanner, D.G., and Mengistu Hulluka, (*Eds.*). Wheat research in Ethiopia: A historical perspective. Addis Ababa. IAR/CIMMYT. *p.* 73-93.

Hoseney, R.C., 1986. Principles of Cereal Science and Technology. *American Association of Cereal Chemists*, St Paul, MN.

Johnson, J.A., and Swanson, C.O, 1942. The testing of wheat quality by recording dough mixer curves obtained from sifted wheat meals. *Cereal Chem.* 19:216-229.

Johnson, V.A. and Marten, P.J., 1987. Wheat, rye and triticale. *In*: Olson, R.A. and Frey, K.J. (*Eds.*). Nutritional quality of cereal grains: Genetic and agronomic improvement. *Agron. Monogram.* 28. ASA, Madison, WI.

Johnson, V.A., Mattern, P.J. and Kuhr, S.L., 1979. Genetic improvement of wheat protein. *In:* Seed protein improvement in cereal and grain legumes; Proceedings of an international symposium, vol 2. IAEA, Vienna. *pp.* 165-179.

Kasarda, D.D., 1989. Glutenin structure in relation to wheat quality. *In:* Pomeranz, Y. (*Ed.*). Wheat is unique. American Association of Cereal Chemists, St. Paul. *pp.* 277-302.

Khatkar, B.S., Bell, A.E. and Schofield, J.D., 1996. A comparative study of the inter-relationship between Mixograph parameters and bread-making qualities of wheat flours and gluten. *J. Sci. Food Agric.* 72:71-85.

Lukow, O.M and McVetty, P.B.E., 1991. Effect of cultivar and environment on quality characteristics of spring wheat. *Cereal Chem.* 68(6):597-601.

MacRitchie, F., 1980. Physicochemical aspects of some problems in wheat research. *In*: Pomeranz, Y. (*Ed.*). Advances in Cereal science and technology, Vol III. American Association of Cereal Technology, St Paul, MN. *pp.*271-326.

MacRitchie, F., 1984. Baking quality of wheat flour. *Adv. Food Res.* 29:201-277.

Martinant, J.P., Nicolas, Y., Bouguennec, A., Popineau, Y., Saulnier, L. and Branlard, G., 1998. Relationship between Mixograph parameters and indices of wheat grain quality. *J. Cereal Sci.* 27:179-189.

NCSS, 2001. Number Cruncher Statistical System, Statistical System for Windows. Jerry L. Hintze. Kaysville, Utah.

O'Brien, L. and Ronalds J.A., 1987. Heritabilities of small scale and standard measures of wheat quality for early generation selection. *Aust. J. Agric. Res.* 38:801-808.

Peterson, C.J., Graybosch, R.J, Baenziger, P.S. and Grombacher, A.W., 1992. Genotype and environmental effects of quality characteristics of hard red winter wheat. *Crop Sci.* 32:98-103.

Peterson, C.J., Graybosch, R.J, Shelton, D.R, and Baenziger, P.S. 1998. Baking quality of hard winter wheat: response of cultivars to environment in the Great Plains. *In:* Braun et al. (*Eds.*). Wheat: Prospects for global improvement, Kluwer Academic Publishers, Netherlands. *pp.* 223-228.

Pomeranz, Y, 1988. Composition and functionality of wheat flour components. In: Pomeranz, Y. (*Ed.*). Wheat: chemistry and technology, vol II. American Association of Cereal Chemists, St. Paul. *pp.* 219-370.

SAS, 1994. SAS/STAT ™. Guide for personal computers. Version 6 Edition. SAS institute Inc., Cary, NC.

Shellenberger, J.A., Pomaranz, Y., Finney, K.F. and Ward, A.B., 1970. Chemical, rheological and bread-making characteristics of selected varieties of various wheat classes. *Baker's Dig.* 44(5):27-31, 34-35.

Singh, M., Ceccarelli, S. and Hamblin, J., 1993. Estimation of heritability from varietal trials data. *Theor. Appl. Genet.* 86:437-441.

Tanner, D.G. and Mwangi, W., 1991. Current issues in wheat research and production in Eastern, Central and Southern Africa. *In:* Tanner, D.G. and Mwangi, (*Eds.*). Constraints and achievements. Seventh regional wheat workshop for Eastern, Central and Southern Africa, Nakuru, Kenya: CIMMYT. *pp.*17-36.

Wikström, K. and Bohlin, L., 1996. Multivariate analysis as a tool to predict bread volume from mixograph parameters. *Cereal Chem.* 73(6):689-690.

Wrigley, C., 2002. The next breakthrough in cereal chemistry-rapid quality testing. *Cereal Foods World.* 47(3):116-118.

Chapter 4 Allelic variation of HMW glutenin subunits in Ethiopian bread wheat cultivars and their quality scores

4.1 Abstract

In a number of studies, high molecular weight glutenins were found to be effective to identify wheat genotypes with good baking quality. The high molecular weight glutenin subunit composition of Ethiopian cultivars and advanced lines was investigated to determine their influence on quality. Three alleles at *Glu-A1*, five at *Glu-B1* and two at *Glu-D1* and eleven different banding patterns were identified. Few cultivars had biotypes at *Glu-A1* and *Glu-D1* loci. There were highly significant differences between genotypes and banding patterns for the SDS-sedimentation test, mixograph development time, alveograph strength and loaf volume but not for protein content. The frequency of subunits 5+10 among genotypes was 73 %. The accumulation of high scoring alleles in Ethiopian germplasm was without deliberate selection pressure towards high *Glu-1* scoring alleles during breeding efforts. Introductions from CIMMYT are widely used and such materials might have gone through selection and accumulation of such alleles at CIMMYT. Most of the studied materials had the required subunits of high quality scores and better values of the quality traits than originally thought. Therefore, the complaints of poor baking quality are unlikely to be due to *Glu-1* allelic variations alone and other environmental and management factors should be investigated.

Key words: Allelic variation, baking quality, HMW-GS, SDS-PAGE, *Triticum aestivum,*

4.2 Introduction

Bread and durum wheat (*Triticum aestivum* L. ssp. *aestivum* and *Triticum turgidum* L. (Thell.) *ssp. durum* (Desf.) Husn.*)* are among the leading cereals in Ethiopia. The country is considered as a primary center of diversity for tetraploid wheats (Vavilov, 1931) and contemporarily it has the greatest diversity. The country is the second largest wheat producer in Eastern, Central and Southern Africa producing about 1,125,000 tons occupying 750,000 hectares (Tanner and Mwangi, 1991; Payne et al., 2001) and is 58 % self sufficient. Bread wheat is expanding in area of production at the expense of traditional durum and emmer wheat crops because of its

broader adaptability and yield advantage (Hailu, 1991; Payne et al., 2001). However, the yield per unit area is low due to influences of various technical and socioeconomic constraints.

Wheat improvement in northwestern Ethiopia is mainly targeted to develop high yielding, widely adapted and disease resistant varieties with inadequate emphasis on grain quality. It is expected that the deterioration of milling and baking quality might occur during efforts to develop varieties for high grain yield (Bingham and Lupton, 1987). The need for improved quality of raw material for the wheat industry is increasing (Tadesse, 2001, survey information, unpublished) and some milling enterprises and traders import grain and flour. Improvement for both reasonable yield and quality must be given higher emphasis in the breeding program to satisfy the growing needs.

A major prerequisite of quality is the presence of intrinsic protein quality. Proteins are useful markers of quality (Payne, 1987). Studies indicate that 20-60 % of the variability between varieties in bread making quality can be accounted for by variation in grain storage proteins (Payne, 1987; Lukow et al., 1989; Kolster et al., 1991; Worland and Snape, 2001). In sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), two groups of reduced subunits of glutenin protein bands are separated: the high molecular weight (HMW) and low molecular weight (LMW) subunits (Payne et al., 1980; Jackson et al., 1983). The HMW glutenin subunits (GS) of wheat protein are quantitatively minor but functionally an important group of gluten proteins in the process of bread making. They are encoded at the Glu-1 loci on the long arm of the group 1 chromosome 1A, 1B, and 1D at *Glu-A1, Glu-B1* and *Glu-D1* loci, respectively (Payne, 1987).

The relationships between HMW-GS and bread making quality were studied as the presence and absence of subunits (Payne et al., 1987) or as the quantity of one subunit related to quality (Ng and Bushuk, 1988; Weegels et al., 1996) and the additivity or combined role of HMW- and LMW-GS in improving bread making quality (Payne et al., 1987; Gupta et al., 1989). Other grain components, such as lipids and carbohydrates, also affect breadmaking quality, possibly by interacting with the gluten proteins. Correlations and genetic studies of HMW-GS (Payne et al., 1987; Pogna and Mellini, 1986) have established subunits with both positive (5+10) and negative (2+12) effects on bread making quality. Other allelic variant pairs showed similar results. In general, a *null* at *Glu-A1* locus, subunit 6+8 encoded at *Glu-B1* and 2+12 at *Glu-D1* are negatively related to the quality parameters (Weegels et al., 1996).

A scoring system for HMW-GS has been developed (Pogna and Mellini, 1986; Payne et al., 1987) as the sum of the contributions of each of the three HMW-GS loci. Many breeding programs have characterized the HMW-GS composition of breeding and released lines/cultivars (Payne et al., 1987; Morguno, 1990; Lukow et al., 1989; Igregas et al., 1999; Nakamura, 2001) in relation to end-use quality and used as a screening test to ensure that good bread making alleles (1, 2*, 7+9, 7+8, 5+10) are incorporated in new cultivars (Lukow, 1991). However, the HMW-GS score has more influence in some sets of wheats than in others (MacRitchie et al., 1990; Bedo et al., 1995). Nevertheless, reference to HMW-GS composition has proved valuable in the segregation of lines in the process of breeding for specific quality targets (Weegels et al., 1996; Cornish et al., 1999) and as indicators of quality when only small amounts of material are available and fast quality prediction is necessary (Weegels et al., 1996).

Selection for these quality alleles will help to reduce the (expensive) cost of extensive field evaluations of large amounts of material, which can sometimes end up with inferior quality. Few lines/varieties selected for quality alleles will be included in the regional variety trials (checked for their improved quality and authenticity). This study investigated the popular commercial and advanced breeding lines from Ethiopia for their HMW protein composition and HMW scores for quality and relationships with some physical measures of quality.

4.3 Material and methods

Thirty wheat genotypes were sampled from two categories: from the widely grown commercial (registered) cultivars and from advanced lines for possible release (Table 9). The advanced lines were in a series of field trials, regional and national, for the last 7-8 years and were selected for their agronomic superiority. The materials were grown in the same environment, in South Africa, to avoid environmental variation and to see real varietal differences. South African cultivars were included for comparison. A RCB design in three replications was used and all agronomic practices were standard for wheat production in the area.

Table 9. List of cultivars and advanced lines used

Line no.	*Cultivar (line)*	*Line no.*	*Cultivar (line)*
1	HAR 2457	16	HAR 1709
2	HAR 1918	17	HAR 1685
3	HAR 2258	18	HAR 604
4	HAR 2348	19	HAR 1522
5	HAR 2566	20	HAR 1775
6	HAR 2807	21	HAR 1868
7	HAR 2029	22	HAR1847
8	HAR 2408	23	HAR 1920
9	HAR 1896	24	HAR 2536
10	HAR 2096	25	HAR 2501
11	HAR 2530	26	HAR 2504
12	HAR 2562	27	HAR 2505
13	HAR 1899	28	HAR 2192
14	K6295-4A	29	Kariega
15	ET13 A_2	30	SST-825

Polyacrylamide gel electrophoresis in the presence of Sodium Dodecyl Sulphate (SDS-PAGE) of glutenins of the seeds was carried out using the procedure of Singh et al. (1991). Six seeds were randomly sampled for each genotype. Each seed was crushed into fine powder. The gliadins were extracted and removed in 70 % ethanol and 50 % *n*-propanol to avoid contamination of glutenins. Glutenins were extracted and reduced from the residue in extraction buffer containing dithiothretol (DTT) and the protein was alkylated using 4-vinylpyridin. Finally, SDS was added and incubated, complexing SDS with the reduced and alkylated glutenin polypeptides. The separating gel of 10 % acrylamide concentration was used for separation and Coomassie brilliant blue R-250 (0.05 *g*) for staining of proteins. The HMW glutenin subunits were identified using the numbering system of Payne and Lawrence (1983). The quality scores were calculated using the methods of Payne et al (1987) and Pogna and Mellini (1986) (Table 10). The materials were studied for their variability in the expression of protein content (FPC, %, AACC 39-11), mixograph development time (MDT, min, AACC 54-, SDS Sedimentation test (SDSS, ml, AACC 56-70), alveograph strength (W, cm^2, AACC 54-30A) and loaf volume (LFV, cm^3, AACC 10-09). The analysis of variance was carried out in general linear model (GLM) of NCSS software (2001) using varieties and banding patterns as factors. Duncan's mean separation (NCSS, 2001) was applied for quality traits showing significant differences between patterns. Rank correlation analysis was calculated between quality parameters.

Table 10. HMW quality scoring methods based on the SDS-sedimentation test and alveograph gluten strength (W) (Belderok et al., 2000)

Locus	Subunit (allele)	Score[b] SDSS[a]	Score[b] W[c]
Glu-A1	2* (b)	3	5
	1 (a)	3	3
	Null (c)	1	2
Glu-B1	17+18 (i)	3	6
	7+8 (b)	3	4
	13+16 (f)	3	6
	7+9 (c)	2	5
	6+8 (d)	1	1
	7 (a)	1	2
	20 (e)	1	1
Glu-B1	5+10 (a)	4	6
	2+12 (d)	2	2
	3+12 (b)	2	2
	4+12 (c)	1	1
Range		3-10	4-17

[b], higher values indicate better quality effects;
[a], Based on Payne et al. (1987); [c], Pogna and Mellini (1986);
( ) = letters in parenthesis are allelic designations

4.4 Results and discussion

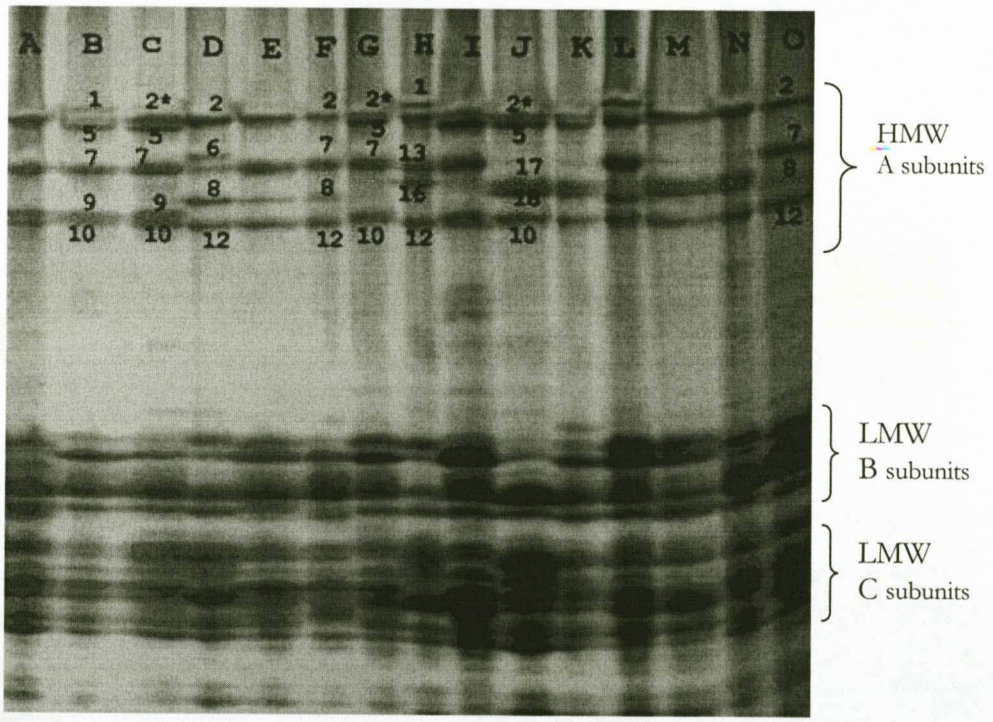
Three alleles at *Glu-A1* locus (a, b and c) of chromosome 1A, five at *Glu-B1* (a, b, c, I and f) of chromosome 1B and two at *Glu-D1* (a and d) of chromosome 1D were identified. Rare alleles were *Glu-A1*c, *Glu-B1*f and *Glu*-B1a. The frequency of each subunit (allele) at the three *Glu*-1 loci is given in Table 11. *Glu*-A1a (2*), *Glu-B1*c (7+9) and *Glu-D1*a (5+10) appeared with a frequency of 74 %, 55 % and 78 %, respectively, as compared to other alleles at each locus. The electrophoresis patterns of selected cultivars along with the standards are presented in Figure 9.

The joint occurrence of different alleles from the three loci is important in the accumulation of scores and also in determining quality (Payne et al., 1987). Twenty-one cultivars had subunits 5+10 and six cultivars had 2+12 at the *Glu-D1* locus (Table 12) and three varieties showed within-line biotypes. Biotypes are groups of individuals with the same genotype within a polymorphic line (Graybosch et al., 1994) identified after analysis of their protein composition. Generally, the presence of subunit 5+10 is considered vital for indication of quality (Payne et al., 1987; Kasarda, 1999) and a maximum score is allocated for its presence. Subunit 5+10 mostly appeared with 7+9 (55 %) and 17+18 (11 %) alleles of *Glu*-B1, which resulted in the

accumulation of high scores for most of the varieties. The widely accepted *Glu-D1d* (2+12) subunits, their presence causing deleterious effects on quality appeared together with subunits *Glu-B1b* (7+8) in three varieties out of six. Twenty six (96 %) of the varieties had five subunits/alleles at the three *Glu-1* loci and no variety had six subunits as one of the allele at *Glu*-A1 was always silent. Some varieties have been reported to have six subunits (Payne and Lawrence, 1983).

Table 11. Frequency of each allele (subunit) at different loci (*Glu-A1, Glu-B1, Glu-D1*)

Glu-A1			*Glu-B1*			*Glu-D1*		
Subunit (Allele)	*No of lines*	*%*	*Subunit (Allele)*	*No of lines*	*%*	*(Allele) Subunit*	*No of lines*	*%*
1 (a)	6	22	7 (a)	2	7	5+10 (a)	21	78
2* (b)	20	74	7+8 (b)	5	19	2+12 (d)	6	22
N (c)	1	4	7+9 (c)	15	55			
			17+18 (i)	4	15			
			13+16 (f)	1	4			



A=HAR1709; B=HAR1522; C=HAR1775; D=HAR2348; E=ET13A$_2$; F &O=Chinese Spring; G=HAR1868; H=HAR2807; I= HAR1685; J= HAR2562; K= HAR604; L=SST-825; M=Kariega;

Figure 9. SDS-PAGE patterns of HMW of some lines and cultivars

Eleven different subunit combinations at the three *Glu-1* loci were identified in the 30 varieties studied, one pattern as a biotype (Table 13). Three varieties were excluded showing mixed

banding patterns due to the presence of biotypes. They showed variation at *Glu-A1* and *Glu-B1* loci. The most frequently appearing pattern was 2*,7+9,5+10 (37 %, alleles b,c,a), found in 11 varieties, followed by 0/2*,7+8,2+12 (13 %, alleles c/b,b,d), observed in three varieties.

Table 12. Jointly occurring subunits of *Glu-B1* and *Glu-D1*

Glu-B1	*Glu-D1*	*No. of lines*	*(%)*
7	5+10	2	7
7+8	5+10	1	4
7+9	5+10	15	55
17+18	5+10	3	11
13+16	2+12	1	4
7+8	2+12	4	15
17+18	2+12	1	4

Among 10 allelic combinations (patterns) at the three loci identified in the studied materials, five of them were represented by single line/cultivar each.

Table 13. Mean of quality traits and *Glu-1* scores of the banding patterns at the three loci

Subunit combination	Alleles	*MDT (min)*	*W (cm^2)*	*SDSS (ml)*	LFV (cm^3)	SDSS score[a]	W Score[b]	Line No
1, 7+8, 5+10	*a,b,a*	2.9	56.4	88.3	994.3	10	13	30
2*,17+18, 5+10	*b,i,a,*	2.8	44.5	79.7	858.7	10	17	12,13,18
2*, 7+9, 5+10	*b,c,a*	2.0	33.8	66.0	827.9	9	16	2,5,7,8,9,10, 16,20,23,24,25,
1, 7+9, 5+10	*a,c,a*	2.0	31.8	68.8	815.9	9	14	1,11,19
2*, 7, 5+10	*b,a,a*	1.9	27.6	77.5	931.5	8	13	17,21
N, 7+9, 5+10	*c,c,a*	1.7	22.2	54.0	733.7	7	13	3
1, 7+8, 2+12	*a,b,d*	2.6	39.1	81.0	887.7	8	9	28
1, 13+16, 2+12	*a,f,d*	1.6	27.6	64.7	846.7	8	11	6
0/2*, 7+8, 2+12	*c/b,b,d*	1.5	25.1	66.7	871.7	8	11	4,14,15
0/2*,17+18, 2+12	*c/b,i,d*	2.6	43.1	91.7	1007.3	8	13	29
1, 7, 5+10	*a,a,a*[†]							

[†]= biotype; [a] =Payne et al, 1987; [b] = Pogna and Mellini, 1986

HMW subunits and patterns with high quality scores were observed in the majority of the cultivars and advanced lines (Table 13). The scores based on SDSS test ranged from 7 to 10. Four of the cultivars had the maximum possible score of 10 i.e., two commercial, one advanced line and one South African included for comparison. Fourteen of the varieties had a score of 9. Generally, 26 out of 27 genotypes (96 %) had a quality score value ranging from 8

to 10 indicating most accumulated higher quality scoring subunits. The three varieties showing biotypes were uniform at the *Glu-D1* locus, had 5+10 subunits (*Glu-D1*d) while one variety was heterogeneous at *Glu-A1* locus. As selection for the 5+10 subunits of *Glu-D1*, for better quality, is the most widely used (Lukow, 1991; MacRitchie et al., 1990; Cornish et al., 1999), the sampled materials were dominated by the same allele (73 %). The accumulation of high scoring alleles in the germplasm was during selection for yield, wide adaptation and disease resistance. No selection pressure towards high *Glu-1* scoring alleles was made in the breeding efforts. Introductions from CIMMYT are widely used and such materials might have gone through selection and accumulation of such alleles at CIMMYT.

The score values using the method of Pogna and Mellini (1986) ranged from 9 to 17; 23 genotypes scored greater than 13 showing higher values which can be associated with quality (Table 13). The low scores were obtained by varieties having null alleles at *Glu*-A1, and single subunit allele at *Glu-B1* combined with 2+12 of *Glu-D1* allele.

The materials were studied for their variability in the expression of protein content (FPC, %), mixograph development time (MDT, min), alveograph strength (W, cm^2) and loaf volume (LFV, cm^3). There was significant variability between genotypes in the quality traits studied except for protein content (Table 13 and 14). The non-significant differences in their protein contents will help to minimize the influence of protein on other parameters of quality.

Table 14. Mean squares of varieties and HMW banding patterns

Source	Varieties		HMW banding patterns	
	Mean squares	F-value	Mean squares	F-value
FPC (%)	1.5	1.52^{NS}	1.4	1.32^{NS}
MDT (min)	1.0	20.9***	1.5	6.5***
SDSS (ml)	374.0	47.5***	603.7	9.09***
W (cm^2)	344.9	27.3***	486.7	6.56***
LFV (cm^3)	16318.4	6.6***	27905.4	6.45***

*** *indicates significant at P<0.001;* ^{NS}*, non-significant*

The genotype mean values of MDT ranged from 1.1-3.2 min, alveograph strength (W) from 15.8 to 60.7 cm^2, and SDSS from 52.7 to 91.7 ml and LFV from 731.3 to 1007.3 cm^3. Three genotypes having the highest possible quality scores i.e., cultivar no. 12, 13 and 18 varied in their MDT, W and SDSS performances. Cultivar 12 was significantly different from the two others in SDSS, MDT and W but not protein. Similar variation was also observed in the rest of

the genotypes having similar banding patterns. It showed that genotypes with similar HMW patterns and high score alleles might not assure similarly higher values of quality parameters as other factors and their interaction are also involved in determining quality (Payne et al., 1987; Gupta et al., 1989). It was also demonstrated that contribution of HMW allelic combination alone in grain quality could range from 20-60 % (Payne et al., 1987; Lukow et al., 1989).

The mean FPC of the subunit patterns ranged from 12.1 to13.5 %, MDT from 1.5 to 2.9 min, SDSS from 54.0 to 91.7 ml and loaf volume from 733.7 to 1007.3 cm^3. To determine the effects of different patterns, an analysis of variance was carried out in GLM of NCSS using subunit combinations as factors. The banding patterns significantly affected the traits studied except protein content (Table 14) showing that selecting for better alleles and their combinations is valuable. The 1st and 3rd highest values of SDSS were achieved by patterns 0/2*,17+18,2+12 and 1,7+8,2+12, respectively. The 2nd and 4th highest values were, respectively, by 1,7+8,5+10 and 2*, 17+18,5+10. To the contrary, Kariega with 0/2*,17+18,2+12 with lower quality score had high SDSS and LFV values and reasonably high values of MDT and W. It was reported that the HMW glutenin score has more influence in some sets of wheats than in others (MacRitchie et al., 1990) and lines with poor banding combinations (2+12) produced a relatively high volume (Bedo et al., 1995). This is likely to be due to the complex interaction of factors that define wheat quality. The two highest MDT and W values were scored by the patterns or allelic combinations having the maximum score of 10 and 5+10 bands at Glu-D1 locus followed by two cultivars holding 2+12 with SDSS score of 8 (Figure 10 and 11). However, there was no significant difference between these four patterns in MDT but pattern 1,7+8,5+10 was significantly different from the rest in W (Table 15). The lowest values in MDT, SDSS and W were achieved by patterns having subunits 2+12. Pattern N,7+9,5+10, with the lowest SDSS score of 7, was a poor performer in all traits indicating the null allele of *Glu*-A1 should be avoided in selection (Payne et al., 1987, Weegles et al., 1996). The three patterns 1,7+8,5+10; 0/2*,17+18,2+12 and 2*,7,5+10 had the highest LFV and their differences were non-significant. There was no significant difference between varieties having values of 800-1007 cm^3. Loaf volume was significantly and positively correlated to SDSS (0.74***), MDT (0.32**) and alveograph W (0.33**). The most valuable allelic combinations in these materials were 1,7+8,5+10 and 2*,17+18,5+10 in MDT, 2*,17+18,2+12 and 1,7+8,5+10 in SDSS, 1,7+8,5+10 and 2*,17+18,5+10 in alveograph W, and 2*,17+18,2+12 and 1,7+8,5+10 in LFV.

Table 15. Mean separation of the subunit combinations

Subunit combination	FPC (%)	MDT (min)‡	SDSS (ml)	W(cm^2)	LFV(cm^3)
1, 7+8, 5+10	12.3	2.9 *a*	88.3 *a*	56.5 *a*	994.3 *a*
2*, 17+18, 5+10	12.4	2.8 *ab*	79.7 *ab*	44.5 *b*	858.7 *bc*
2*, 7+9, 5+10	12.6	2.0 *bc*	66.0 *bc*	33.8 *bcd*	827.9 *bcd*
1, 7+9, 5+10	12.1	2.0 *bc*	68.8 *bc*	31.8 *bcd*	815.9 *bcd*
2*, 7, 5+10	12.1	1.9 *bc*	77.5 *ab*	27.6 *bcd*	931.5 *ab*
N, 7+9, 5+10	13.2	1.7 *c*	54.0 *c*	22.2 *d*	733.7 *d*
1, 7+8, 2+12	12.6	2.6 *ab*	81.0 *ab*	39.1 *bcd*	887.7 *bc*
1, 13+16, 2+12	12.7	1.6 *c*	64.7 *bc*	27.6 *cd*	846.7 *bcd*
0/2*, 7+8, 2+12	13.5	1.5 *c*	66.7 *bc*	25.1 *cd*	871.7 *bc*
0/2*,17+18, 2+12	12.6	2.6 *ab*	91.7 *a*	43.1 *bc*	1007.3 *a*
mean	12.6	2.2	73.8	35.1	877.5

‡=*values with similar letters are not significantly different at P< 0.05*

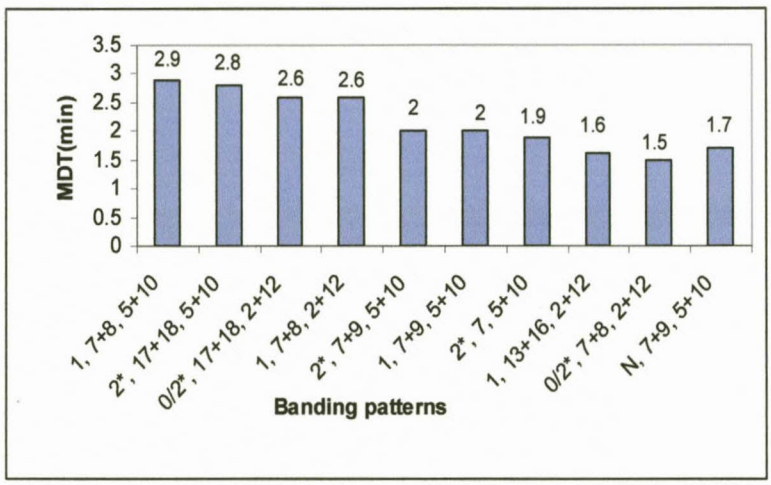


Figure 10. Mixograph development time (MDT) of 10 banding patterns

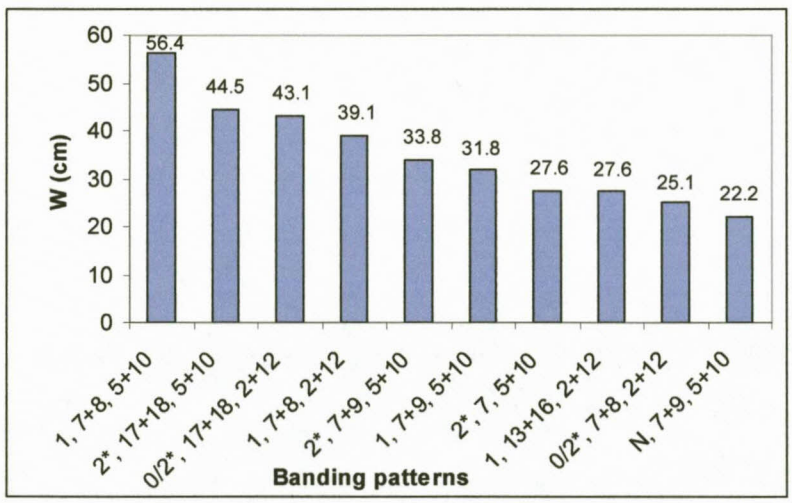


Figure 11. Gluten strength (W) values of 10 banding patterns

4.5 Conclusion

In cultivars/lines studied, varieties having subunits 5+10 were more frequent and generally gave better quality results. Genotypes having similar patterns had variable expressions in the studied traits. There were also genotypes with subunits 2+12 in their patterns which gave better results like cultivars Kariega and HAR 2192. Avoiding the null allele at *Glu-A1* is important. For better results, breeding and selection using HMW glutenin subunits and *Glu-1* scores (Wrigley, 2002) combined with one or more direct physical (MDT or W) and indirect (SDSS, protein content) measurements of quality (Dong et al., 1992) in parents and progenies will have critical importance. Since higher yields are the main objective of the breeding program, strategies for medium-high grain yielding with acceptable quality is necessary and evaluation of quality at all stages of agronomic evaluation should be designed. It was shown that most of the varieties had the required *Glu-1* alleles and high values of studied physical measures of quality as opposed to what was originally thought. Therefore, this study has confirmed that complaints of poor baking quality are unlikely to be due to Glu-1 allelic variations alone. However, the current study was a preliminary investigation of the trends of HMW-GS in cultivars/lines of northwestern Ethiopian breeding program. Further studies must be carried out to establish the contribution of HMW-GS and other factors affecting quality such as LMW-GS, gliadins protein components, environmental and management aspects. Many researchers have revealed that LMW-GS have influences in dough quality in bread wheat (Gupta et al., 1994; Cornish et al., 1999). It was also suggested that quality would be more accurately assessed if LMW-GS are considered in conjunction with the HMW-GS (Gupta et al., 1994).

4.6 References

AACC (American Association of Cereal Chemists), 2000. Approved methods of the AACC. 10th edition. The association: St. Paul, MN.

Bedő, Z., Kárpáti, M., Vida, G., Kramarik-kissimon, J. and Láng., 1995. Good bread making quality wheat (*Triticum aestivum*, L.) genotypes with 2+12 subunit composition at the Glu-D1 locus. *Cereal Res. Commun.* 23(3):283-289.

Belderok, B., Mesdag, J. and Donner, D.A., 2000. Bread making quality of wheat: A century of breeding in Europe. Kluwer Academic Publishers, Netherlands. *p.* 261.

Bingham, J. and Lupton, F.G.H., 1987. Production of new varieties: an integrated research approach to plant breeding. *In*: Lupton, F.G.H. (*Ed.*). Wheat breeding, its scientific basis. Chapman and Hall, London and New York. *pp.* 487-538.

Cornish, G.B., Panozzo, J.F. and Wrigley, C.W., 1999. Victorian wheat protein families. *In*: O'Brien, L. and Blakeney, A.B. Ross, A.S. and Wrigley, C.W. (*Eds.*). Cereals 98. Proc. 48th Australian Cereal Chemistry Conf., RACI: Melbourne, Australia. *pp.* 183-188.

Dong, H., Sears, R.G., Cox, T.S., Hoseney, R.C., Lookhart, G.L. and Shorgen, M.D., 1992. relationship between protein composition and Mixograph and loaf characteristics in wheat. *Cereal Chem.* 69(2):132-136.

Graybosch, R.A, Peterson, C.J., Lee, J-H. and Shelten, D.R., 1994. Effect of glutenin protein polymorphism on breadmaking quality of winter wheats. *Crop Sci.* 34: 628-635.

Gupta, R.B., Singh, N.K. and Shepherd, K.W., 1989. The cumulative effect of allelic variation in LMW and HMW glutenin subunits on physical dough properties in progeny of two bread wheats. *Theor. Appl. Genet.*77:57-64.

Gupta, R.B. and MacRitchie, F., 1994. Allelic variation at glutenin subunit and gliadin loci, Glu-1, Glu-3 and Gli-1 of common wheats. II. Biochemical basis of the allelic effects on dough properties. *J. Cereal Sci.* 19:19-29.

Hailu Gebre-Mariam, 1991. Bread wheat breeding and genetics in Ethiopia. *In:* Hailu Gebre-Mariam, Tanner, D.G. and Mengistu Hulluka, (*Eds.*). Wheat research in Ethiopia: A historical perspective. Addis Ababa. IAR/CIMMYT. *pp.* 73-93.

Igregas, G., Guedes-Pinto, H., Carnide, V. and Branlard, G., 1999. The high and low molecular weight glutenin subunit and ω-gliadin composition of bread and durum wheats commonly grown in Portugal. *Plant Breed.* 118:297-302.

Jackson, E.A., Holt, L.M. and Payne, P.I., 1983. Characterization of high-molecular-weight gliadin and low-molecular-weight glutenin subunits of wheat endosperm by two-dimensional electrophoresis and chromosomal localization of their controlling genes. *Theor. Appl. Genet.*66:29-37.

Kasarda, D.D., 1999. Glutenin polymers: The in vitro to in vivo transition. *Cereal Foods World* 44:566-571.

Kolster, P., van Eeuwijk, F.A. and van Gelder, W.M.J., 1991. Additive and epistatic effects of allelic variation at the high molecular weight glutenin subunit loci in determining the bread-making quality of breeding lines of wheat. *Euphytica* 55:277-285.

Lukow, O.M., 1991. Screening of bread wheats for milling and baking quality- a Canadian perspective. *Cereal Foods World* 36(6):497-501.

Lukow, O.M., Payne, P.I. and Tkachuk, R., 1989. The HMW glutenin subunit composition of Canadian wheat cultivars and their associations with bread making quality. *J. Sci. Food Agric.* 46:451.

MacRitchie, F., du Cros, D.L. and Wrigley, C.W., 1990. Flour polypeptides related to wheat quality. *Adv. Cereal Sci. Technol.* 10:79-145

Morguno, A.I., Rogers, W.J., Sayers, E.J. and Metakovsky, E.V., 1990. The high molecular weight glutenin subunits of Soviet grown varieties. *Euphytica* 51:41-52.

Nakamura, H., 2001. Genetic diversity of high-molecular-weight glutenin subunits compositions of in landraces of hexaploid wheat from Japan. *Euphytica* 120:227-234.

Ng, P.K.W. and Bushuk, W., 1988. Statistical relationship between high molecular weight subunits of glutenin and bread making quality of Canadian grown wheats. *Cereal Chem.* 65(5): 408-413.

Payne P.I. and Lawrence G.J., 1983. Catalogue of alleles for the complex gene loci, Glu-A1, Glu-B1 and Glu-D1 which code for high molecular weight sub units of glutenins in hexaploid wheat. *Cereal Res. Comm.* 11:29-35.

Payne, P.I., Law, C.N. and Mudd, E.E., 1980. Control by homoeologous group 1 chromosomes of the high-molecular-weight subunits of glutenin, a major protein of wheat endosperm. *Theor. Appl. Genet.*58:113-120.

Payne, P.I., 1987. Genetic of wheat storage proteins and the effect of allelic variation on bread making quality. *Annual reviews in plant physiology* 8:141-153.

Payne, P.I., Mark A.N., Krattiger A.F. and Holt L.M., 1987. The relationship between HMW glutenin subunits composition and the bread making quality of British-grown wheats. *J. Sci. Food Agric.* 40:51-65.

Payne, T.S, Wanjama, J.K and Girma, B, 2001. Eastern African wheat pool. *In*: Bonjean, A.P. and Angus, W.J. (*Eds.*). The wheat world book: A history of wheat breeding. London: Intercept.

Pogna, N.E. and Mellini, F., 1986. Alla ricerca delle basi biochemiche e genetiche della qualita del glutine. *Inform Agr.* 42:65-66.

Singh N.K., Shepherd, K.W. and Cornish G.B., 1991. A simplified SDS-PAGE procedure for separating LMW subunits of glutenins. *J. Cereal Sci.* 14:203-208.

Tanner, D.G. and Mwangi, W., 1991. Current issues in wheat research and production in Eastern, Central and Southern Africa. *In:* Tanner, D.G, and Mwangi, (*Eds.*). Constraints and achievements. Seventh regional wheat workshop for Eastern, Central and Southern Africa, Nakuru, Kenya: CIMMYT. *pp.*17-36.

Vavilov, NI, 1931. The wheat of Abyssinia and their place in the general system of wheats, Bulletin of Applied Botany, Genetics, and Plant Breeding, Supplement 51, *p.* 233.

Weegels, P.L, Hamer, R. J. and Schofield, J.D., 1996. Functional properties of wheat glutenin. *J. Cereal Sci.* 23: 1-18.

Worland, T. and Snape, J.W., 2001. Genetic basis of worldwide wheat varietal improvement. *In*: Bonjean, A.P. and Angus, W.J. (*Eds.*). The world wheat book: A history of wheat breeding. London: Intercept. *pp.* 84-85.

Wrigley, C., 2002. The next breakthrough in cereal chemistry-rapid quality testing. *Cereals Food World* 47(3):116-118.

Chapter 5 Combining ability analysis for quality traits in crosses of bread wheat (*Triticum aestivum* L.) cultivars tested in northwestern Ethiopia

5.1 Abstract

A diallel trial was conducted at two environments. Data on indirect and direct measurements of quality were analyzed. There was significant variation between parents and all genotypes for most traits studied. The general combining ability (*GCA*) effects of almost all traits were significant. All traits had significant *GCA* effects in the combined analysis. The specific combining ability (*SCA*) effect was significant for few traits; the significant *GCA* effect was much higher for the same traits indicating the predominance of additive over dominance gene effects. The *GCA: SCA* ratio ranged from 0.7 to 37. Its magnitude was lower in lower yielding environments. The traits that interacted with environment also showed significant interaction in combining abilities. The interaction was predominantly *GCA* by environment for hectoliter weight, flour yield (FLY), SDS-sedimentation (SDSS) test and vitreous kernels (VK). Hectoliter weight and flour yield are the only traits which showed *SCA* by environment interaction indicating some specific crosses were sensitive to testing sites for the same traits. Grain yield correlated negatively with protein content. Highly heritable traits were mixograph development time (MDT), SDSS, hardness index, grain weight, FLY and protein content. The range of positive heterosis was 0-11 % for most traits. In these sets of parents, utilizing the additive genetic variance would be effective to improve *MDT*, *SDSS*, *FLY*, *VK* and thousand-grain weight. The similarities of mean rankings, positive rankings for *GCA* effects, heritabilities and correlations for the most important traits between the two locations will facilitate the use of one of the two locations for preliminary evaluation and selections.

Key words: Bread wheat, combining ability, diallel analysis, GCA, SCA, wheat quality

5.2. Introduction

Bread wheat (*Triticum aestivum* L.) is the leading food crop in the world and mostly used for pan bread and related products. Its end uses depend upon the inherent quality and quantity of gluten proteins, specific to bread wheat, and other factors related to growing conditions;

overall quality depends on many genetic and non-genetic factors. To improve wheat quality understanding of the inheritance of wheat quality traits, joint inheritance and their association with the environment is vital (Baker and Sutherland, 1991). Combining ability analysis, general (*GCA*) and specific (*SCA*), of diallel crosses are useful to understand the nature of gene action involved in determining quantitative traits (Griffing, 1956a) and to identify crosses with superior performance for use in a practical breeding program. The *GCA* effects represent the fixable component of genetic variance. The *SCA* represents a non-fixable component of genetic variation, related to heterosis.

It has been found that progress in breeding for grain yield has been slower in the high quality bread making varieties (Bingham and Lupton, 1987). This may partly be attributed to the logistical difficulties of selecting for the many additional independently inherited characters of a top quality variety. Edwards (1987a) observed that GCA is of greater importance than SCA in wheat, although the latter appears to be more important in space plantings. Many reports suggest that GCA is the principal source of improved grain yield in hybrids, as in line varieties (Borghi et al., 1989; Morgan et al., 1989, Pickett, 1993) with small contribution from SCA. Perenzin et al. (1987) and Edwards (1987b) reported mean test weights that equal or are above the means of parents. Crosses between parents of different texture have been found to produce variable results (Rodriguez et al., 1967; Edwards, 1987a). Studies on flour extraction levels in wheat crosses vary from no heterosis (Bitzer and Fu, 1972), intermediate (Edwards, 1987a) and higher levels than parents.

There are several quality tests, which examine the rheologcal and baking properties of flour (Blackman and Payne, 1987). SDSS is widely used to indicate protein quality. It was correlated with dough strength measures, and it was suggested as a useful test to select for dough strength (O'Brien and Ronnalds, 1987). Brears et al. (1989) reported that most hybrids were intermediate in sedimentation volume. Quantity of protein is largely the result of environment and has been shown to be under polygenic control. Protein levels in hybrids have been reported to lie between parents, fall near to the lower parent and lower than the high parent. Higher values can therefore be achieved by selection of parents (Stuber et al., 1962). Matuz et al. (1993) found negative heterosis or intermediate inheritance for water absorption, development time, stability of dough, loaf volume and protein content. Borghi et al (1987) found crosses to be lower in dough quality than parents, sometimes approaching the lower parent. Edwards (1987b) reported that hybrids were generally intermediate in dough characteristics although there were exceptions in both directions. Most researchers have

concluded that breeding for bread making quality is possible although it will be necessary to select parents carefully to achieve this. According to a review of Pickett (1993), it appears that bread wheat quality is cross specific.

Genetic studies involving selected and adapted parents need more attention, because such crosses are expected to offer desirable genetic variabilities. In addition, multi-location testing of diallel trials is necessary to obtain reliable genetic information since interactions of genetic effects (*GCA*, *SCA*, genetic variances) with environments are significant and should be addressed (Beck et al., 1991). The objectives of this study were to investigate the combining ability, the relative importance of additive and non-additive gene effects, genetic associations and the inheritance of grain and flour quality traits in wheat crosses of adapted cultivars and selected lines at different environments.

5.3 Material and methods

Six cultivars were crossed in all possible combinations according to method II of Griffing (Griffing, 1956b). The six parents and their 15 F_1's were grown in a RCB design with three replications at two agro-climatically different locations (Adet and Motta) in Ethiopia. The plot area was two m^2 consisting of four rows of 2.5 m length. The inter and intra row spacing were 20 cm and 10 cm, respectively. The plots were fertilized with 92/46 kg ha^{-1} of N/P_2O_5, respectively, in the form of urea/DAP. The unpredicted incidence of hybrid necrosis (dwarfness) was encountered in all crosses with one parent, Snack. The F_1 progeny of these cross combination were unable to set seeds (Pfeffer and Zeller, 1987), died prematurely and crosses with Snack were rejected from the diallel.

The methods and characters measured were grain yield (g), thousand grains weight (g), hectoliter weight (kg hl^{-1}), flour yield (Junior Quadromat mill, %), flour protein (AACC (2000) 39-11, %), mixograph development time (AACC 54-40A, min), SDS sedimentation test (AACC 56-70, ml), falling number (AACC 56-70, sec), and vitreous kernels (sliced and counted, %) were collected. Single kernel measurements i.e., hardness index, grain diameter (mm) and weight (mg) were taken using the Single Kernel Characteristics System (SKCS) (AACC 53-31). Bread loaf volume (AACC 10-09, cm^3) and alveograph, P/L ratio (P/L) and strength (W, cm^2) measurements (AACC 54-30A) were determined from the blended samples of the three replications for each environment.

The analysis of variance and combining ability analysis were done using the DIALLEL-SAS program described by Zhang and Kang (1997). Mixed effects model was used with genotypes as fixed, and locations and replications as random (Griffing, 1956b; Dhillon et al., 1990). Based on the expectation of mean square (Table 16), estimates of *GCA* and *SCA* variances and their interactions with the environment were obtained for each trait. The appropriate variance components i.e., additive genetic variance (σ^2_A), additive by environmental variance (σ^2_{AE}), dominance genetic variance (σ^2_D), dominance by environmental variance (σ^2_{DE}) and error variance (σ^2_e) for individual and across location(s) from the expectation of mean squares (Griffing, 1956b; Sughroue and Hallaur, 1997) were computed as: $2(\sigma^2_{GCA}) = \sigma^2_A$; $2(\sigma^2_{GCAxE}) = \sigma^2_{AE}$; $\sigma^2_{SCA}=\sigma^2_D$; $\sigma^2_{SCAxE}=\sigma^2_{DE}$; The narrow ($h^2_n$) sense heritability was calculated for each character for individual and combined environments as $h^2_n = \sigma^2_A / (\sigma^2_A+\sigma^2_D+\sigma^2_{AE}+\sigma^2_{DE}+\sigma^2_E)$. Spearmans' rank correlations for GCA estimates were computed to assess the significance of genetic correlation of breeding values (Falconer and Mackay, 1996). The mid parent advantage (*MA*), the increase of a hybrid for a given character above the mean of its' parents (*MP*), and heterosis (*H*), the increase of a hybrid above the better parent (HP) for each character was calculated (Morgan, 1998) as: $MA = \left(\frac{F1 - MP}{MP}\right) * 100$; $H = \left(\frac{F1 - HP}{HP}\right) * 100$

Table 16. ANOVA giving expectation of mean squares

		Mean Square expectations	
Source	MS	Multiple environment	Single environment
GCA	MS_G	$\sigma^2_e + k(n+2)\sigma^2_{GE} + kl(n+2)\phi^2_G$	$\sigma^2_e + \sigma^2_S + k(n+2)\phi^2$
SCA	MS_S	$\sigma^2_e + k\sigma^2_{SE} + kl\phi^2_S$	$\sigma^2_e + \phi^2_S$
GCA x E	MS_{GE}	$\sigma^2_e + k(n+2)\sigma^2_{GE}$	
SCA x E	MS_{SE}	$\sigma^2_e + k\sigma^2_{SE}$	
Error	MSe	$\sigma^2_e +$	σ^2_e

K, replication; l, location; n, number of parents; E, environment; σ^2 and ϕ^2, variance components due to random and fixed effect respectively; MS, mean square

5.4 Results and discussion

The parents were significantly different in 1000-grain weight (TGW), flour yield (FLY), mixograph development time (MDT), SDS-Sedimentation volume (SDSS), vitreous kernels (VK), hardness index of single kernel characterization system (SKHI), seed diameter of single kernel characterization system (SKDM) and seed weight of single kernel characterization system (SKWT) (Tables 17 and 18).

SST 57 had consistently higher values of MDT, SKHI and VK. The high protein parent was SST 825 while Houtman had the highest hectoliter weight (HLW). Kariega out performed the other parents at both environments in grain yield (GYD), 1000-grain weight (TGW), SDSS, SKDM and SKWT. This was supported by the highly significant variability found among entries in most characters except GYD and TGW at the highest mean yielding location, Adet, and GYD, FLY and SKDM at the lowest yielding site, Motta (Table 18).

Table 17. Mean yields and quality parameters of parents and their F1's across environments (Adet*Motta)

PN & crosses	GYD (g/plot)	TGW (g)	HLW (kg hl^{-1})	FLY (%)	FPC (%)	MDT (min)	SDSS (ml)	VK (%)	SKHI	SKDM (mm)	SKWT (mg)	W P/L	W (10^{-4} J)	LFV (cm^3)
1	503.6	36.8	76.3	64.7	9.6	2.5	72.7	46.7	31.7	2.4	35.8	0.3	18.7	1000
2	419.1	29.2	75.2	60.4	9.2	3.8	54.3	71.0	56.6	2.2	28.6	0.6	25.7	982
3	317.0	29.4	75.1	62.0	10.4	2.8	48.7	67.3	46.3	2.0	28.4	0.3	18.2	952
4	496.4	31.8	74.3	62.6	8.6	2.4	42.5	58.7	46.2	2.1	32.0	0.7	12.9	958
5	444.2	31.0	76.6	61.8	9.6	2.6	60.0	61.7	49.5	2.1	29.9	0.5	19.2	966
4x5	555.7	33.3	75.3	62.6	8.6	2.4	45.8	57.7	44.5	2.1	32.0	0.7	13.8	954
3x5	390.3	30.4	75.2	62.2	9.8	2.7	55.3	62.7	46.1	2.0	28.6	0.4	14.5	944
2x5	406.5	31.4	73.7	60.2	9.6	3.0	59.7	70.3	48.8	2.1	30.1	0.6	21.0	954
1x5	447.6	31.8	75.1	62.1	9.2	2.5	62.3	54.3	40.8	2.2	32.5	0.5	16.4	944
3x4	392.3	31.5	72.9	61.0	9.1	2.5	45.5	55.0	39.2	2.0	29.7	0.4	12.3	958
2x4	395.2	30.9	73.7	60.5	8.9	3.2	49.3	71.3	50.0	2.1	29.7	0.6	16.2	926
1x4	494.2	34.6	74.6	63.4	8.3	2.5	48.5	48.0	32.9	2.2	33.3	0.4	10.7	972
2x3	426.7	32.6	75.5	60.9	9.5	3.3	53.3	69.0	45.5	2.1	29.2	0.4	15.9	894
1x3	401.8	32.1	75.0	62.9	9.2	2.7	57.7	50.0	33.7	2.1	31.1	0.3	14.1	964
1x2	429.7	32.1	74.4	61.9	9.1	3.5	61.7	59.3	39.5	2.2	31.6	0.4	17.7	1002
Gra. mean	434.7	31.9	74.9	61.9	9.2	2.8	54.5	60.2	43.4	2.1	30.8	0.5	16.5	958
Mean Adet	466.0	28.8	73.4	61.7	10.6	2.9	64.2	69.3	47.1	2.0	27.9			
Mean Motta	403.4	35.0	76.3	62.2	7.9	2.7	44.7	51.1	39.8	2.3	33.8			
CV (%)	23.6	8.3	1.1	2.3	4.8	9.2	9.1	12.4	11.8	6.2	7.1			
LSD(0.05)	99.2	2.6	0.8	1.4	0.4	0.2	4.8	7.2	5.0	0.1	2.1			

PN=parent number, 1=Kariega, 2=SST 57; 3=SST 825; 4=Houtman; 5=Cracker

In a combined analysis, there was significant genotypic and environmental variation. However, flour protein content (FPC), HLW and VK were the only traits sensitive to environmental

changes. The falling number values were ≥410 sec for all plots; the analysis was stoped after 410 sec assuming all grains were sound. The presence of significant variation between genotypes, parents and their crosses, in most characters permitted the analysis of combining abilities (Griffing, 1956b).

Table 18. Mean squares for yield and quality traits at individual and pooled environment (s)

	Source of variation					
	Entry (parents &crosses)[†]					Only Parents
Traits	Adet	Motta	Combined	Env	Entry x Env	(Combined)[§]
GYD	26666.3[NS]	9203.9	20744.3*	88247.2**	15125.9	34106.9[NS]
TGW	16.2 [NS]	14.8***	22.2***	864.3***	8.8 [NS]	56.8*
HLW	6.7***	5.7***	5.8***	193.3***	6.7***	5.3[NS]
FLY	10.2***	2.1 [NS]	9.0***	5.6 [NS]	3.3 [NS]	14.3*
FPC	1.2***	0.8**	1.6***	168.9***	0.04*	2.4[NS]
MDT	0.6***	0.6***	1.1***	0.4**	0.1 [NS]	1.9***
SDSS	295.0***	142.2***	389.4***	8565.4***	47.8*	797.9**
VK	110.2***	468.1***	422.9***	7434.7***	155.4**	527.1*
SKHI	158.5***	165.0***	296.8***	1209.4***	26.7 [NS]	492.4***
SKDM	0.05**	0.03 [NS]	0.05***	1.7***	0.03 [NS]	0.1*
SKWT	19.9***	12.4*	25.5***	790.7***	6.9 [NS]	56.5**
P/L [‡]			0.04*			
W[‡]			29.3***			
LFV[‡]			1450.3 [NS]			

,,*** indicates significant at P<0.05, P<0.01 and P<0.001, respectively; [NS], non-significant; Env, environment; [‡], estimated from blended samples; [†]= Degrees of freedom for Entry, 14; environment, 1; Entry x Env, 14; Error, 56; [§] = Degree of freedom for parents, 4; Error,25*

The mean squares of *GCA* of all measured traits were significant except TGW at Adet, and GYD and FLY at Motta indicating most traits are controlled by additive gene action (Table 19). The *SCA* was significant for HLW and FPC at both locations, SDSS and *VK* only at Adet but the magnitude of significant *GCA*, *GCA*: *SCA* was much higher for the same traits, which showed the predominance of additive gene effects. Those traits, which interacted with the environments also showed significant *SCA* by environment, *SCA x E*, or *GCA* by environment interaction, *GCA x E*. *GCA* x E was observed for HLW, FLY, SDSS and vitreous kernels. The magnitude of *GCA* effects was reduced in the low yielding environment (Motta) while MDT, SDSS, SKHI, SKWT maintained high *GCA*: *SCA* in both environments i.e., the exploitation of *GCA* might be high in high potential environments. HLW was the sole trait that was significant for *GCA*, *SCA*, and *GCA* x E and *SCA* x E. It also had the lowest GCA: SCA ratio, less than unity at Adet.

Table 19. Mean squares of combining abilities for separate and combined environments

Traits	GCA†			SCA			GCA x Env	SCA x Env	GCA:SCA		
	Adet	Motta	Pooled	Adet	Motta	Pooled			Adet	Motta	Pooled
GYD	20152.1*	3335.4	49253.4**	4383.4	2961.0	9340.7 ^{NS}	21209.1 ^{NS}	12692.7 ^{NS}	4.6	1.1	5.3
TGW	7.8^{NS}	12.4***	53.18***	4.5 ^{NS}	2.0	9.9 ^{NS}	7.3 ^{NS}	9.4 ^{NS}	1.7	6.3	5.4
HLW	1.7***	2.6***	7.813***	2.4***	1.6***	4.9***	5.1***	7.3***	0.7	1.6	1.6
FLY	10.1***	1.2 ^{NS}	25.9***	0.7 ^{NS}	0.5 ^{NS}	2.3 ^{NS}	7.9**	1.4 ^{NS}	13.9	2.5	11.5
FPC	1.2***	0.4***	4.3***	0.1**	0.2*	0.5**	0.4 ^{NS}	0.4*	11.2	1.9	7.8
MDT	0.7***	0.6***	3.7***	0.0 ^{NS}	0.0 ^{NS}	0.1 ^{NS}	0.1^{NS}	0.1 ^{NS}	35.3	17.7	36.9
SDSS	297.5***	137.3***	1233.2***	18.7***	11.4 ^{NS}	51.9*	71.3*	38.3 ^{NS}	15.9	12.0	23.8
VK	80.6***	436.3***	1292.9***	19.2*	43.9 ^{NS}	75.0 ^{NS}	257.8**	114.4 ^{NS}	4.2	9.9	17.2
SKHI	174.2***	129.5***	901.6***	4.3 ^{NS}	25.2 ^{NS}	54.9*	9.4 ^{NS}	33.6 ^{NS}	40.5	5.1	16.4
SKDM	0.04***	0.02*	0.2***	0.0 ^{NS}	0.0 ^{NS}	0.0 ^{NS}	0.0 ^{NS}	0.0 ^{NS}	5.7	4.2	18.3
SKWT	19.4***	9.6***	83.4***	1.6 ^{NS}	2.0 ^{NS}	2.3 ^{NS}	3.4 ^{NS}	8.3 ^{NS}	12.4	4.8	35.8
P/L‡			0.06**			0.01 ^{NS}					6.0
W‡			36.3***			5.8**					6.3
LFV‡			927.1 ^{NS}			644.3 ^{NS}					1.4

,,*** indicates significant at P<0.05, P<0.01 and P<0.001, respectively; ^{NS}, non-significant; † = Degrees of freedom for GCA, 4; SCA, 10; GCA x Env, 4; SCA x Env, 10; Error, 56; ‡,estimated from blended samples; DF, degrees of freedom; GCA, general combining ability; SCA, Specific combining ability; Env, environment*

At Adet, Houtman and Kariega had the highest positive *GCA* effects for grain yield and low but positive effects for Cracker (Table 20). They had positive but non-significant SCA effects between them, Houtman x Cracker and Kariega x Houtman. Thousand grain weight, FLY, MDT, SKHI, SKDM and SKWT had non-significant dominance genetic variances. Kariega contributed the highest positive gene effects for TGW (1.5), FLY (1.7), SKDM (0.1) and SKWT (2.5). The highest contribution of positive gene effects for MDT (0.5) and SKHI (5.2) was from SST 57. The two high mean protein parents varied in their SCA effects depending on their combinations. Generally, the highest and lowest grain yielding hybrids/parents, respectively, had the lowest and highest SCA gene effects for protein. The crosses with Kariega had higher mean SDSS values than the rest of the crosses. There was a significant contribution of genes with negative effects for this character, which showed a reduction of its values in some combinations (Houtman x Cracker and Kariega x Houtman). Cross SST 57 x Cracker had the highest positive SCA effect for VK (5.2).

Table 20. General combining ability effect of parents at individual locations

Location	PN	GYD (g plot^{-1})	TGW (g)	HLW (kg hl^{-1})	FLY (%)	FPC (%)	MDT (min)	SDSS (ml)	VK (%)	SKHI	SKDM (mm)	SKWT (mg)
ADET	1	28.3	1.5	0.7	1.7	-0.2	0.0	7.8	-5.0	-7.8	0.1	2.5
	2	-37.6	-0.4	-0.5	-1.6	0.1	0.5	1.7	3.5	5.2	0.0	-1.2
	3	-68.3	-0.9	0.3	-0.3	0.5	0.0	-2.1	1.3	-0.3	-0.1	-1.6
	4	67.3	0.7	-0.5	0.5	-0.6	-0.3	-9.8	-1.8	-0.4	0.0	0.8
	5	10.2	-0.9	0.0	-0.3	0.2	-0.2	2.4	2.0	3.3	0.0	-0.5
SE Gi		24.1	0.6	0.2	0.3	0.1	0.0	0.8	1.0	0.6	0.0	0.4
Gi-Gj		38.1	1.0	0.3	0.4	0.1	0.1	1.3	1.5	1.0	0.0	0.7
MOTTA	1	20.9	2.1	0.1	0.6	0.1	-0.2	6.0	-11.0	-6.5	0.1	1.8
	2	5.6	-1.3	0.1	-0.5	0.0	0.5	-0.1	11.0	5.2	0.0	-0.9
	3	-35.5	-0.9	-0.4	0.1	0.3	0.0	3.0	1.6	-0.7	-0.1	-1.2
	4	-3.9	0.0	-0.7	-0.2	-0.4	-0.2	-5.3	-1.6	0.0	0.0	0.2
	5	12.8	0.1	0.9	0.0	0.1	-0.1	2.3	0.0	2.1	0.0	-0.5
SE Gi		14.9	0.4	0.1	0.3	0.1	0.1	1.1	1.8	1.3	0.0	0.4
Gi-Gj		23.7	0.6	0.2	0.5	0.2	0.1	1.7	2.9	2.0	0.0	0.7

SE, standard error; Gi, critical difference to test the significance of GCA effects of a parent; Gi-Gj, critical difference to test the significance of GCA effects of selected parents

At Motta, genotypic differences were significant for all characters except for GYD, FLY and SKDM). It was the lowest protein (7.3 to 9 %) and grain-yielding environment. The other parameters with low mean ranges include SDSS, VK and SKDM. Mixograph development time and FLY maintained similar mean ranges at both locations. Most parameters showed additive gene effects except HLW and FPC, which showed dominant gene effects. All combinations with the high HLW parent, Kariega, had significant negative *SCA* effects (-0.5 to −1.1). Parent SST 57 contributed the significantly highest negative and positive SCA gene effects depending on its combinations. Eight out of 10 crosses showed negative *SCA* effects for protein and the same crosses had positive *SCA* effects for grain yield showing similar protein/yield gene action trend. However, only Kariega x SST825 had a significant negative gene effect (-0.8) for protein. This cross was derived from a high mean protein parent, SST825, at this location indicating high protein parents might not necessarily give high protein additive gene effects. The significant additive gene action of most quality traits in this environment will allow breeders to improve most of these characters by using parents with high *GCA* effects. The low stand establishment and the space advantage has led kernel measurements such as TGW, HLW, SKDM and flour yield to be higher at the low yielding site, Motta. In a combined analysis, all traits showed significant additive genetic variance, except loaf volume. Significant dominance gene effects were observed only for HLW, FPC, SDSS, W and SKHI as it was for separate environments. Therefore, additive and non-additive gene actions were consistent in both individual and combined environments for most traits.

Various researchers have reported the prevalence of GCA effects over SCA in the control of most traits studied (Edwards, 1987a; Morgan et al., 1989; Le Gouis et al., 2000). The largest negative (-52) and positive (32) *GCA* effect of GYD was observed for SST 825 and Houtman, respectively (Table 21). However, the negative *SCA* variance of Houtman explained that its high *GCA* effect was transmitted differently depending on the specific combination, the highest effect (76) was for Houtman x Cracker. No negative *GCA* effect was desired for grain yield but two parents having the highest negative *GCA* effect (SST 57 and SST 825) combined to produce the 2nd highest increment of *SCA* effects (60^{NS}) in yield. The two parents having positive GCA effects for TGW, Kariega and Houtman, similarly had positive gene effects for GYD, FLY and SKWT showing association in their effects. All hybrids scored below 10.5 % mean protein content across these environments. Generally, the low protein could be associated with the prevalence of leaf diseases during grain filling. The presence of significant non-additive gene action and its interaction with environment for protein lead to select specific advantageous crosses for specific environments. The mixograph value appeared entirely controlled by additive gene effects and had the highest *GCA*: *SCA* and did not interact with the environment. The highest average positive significant additive gene effect of MDT was for parent SST 57 (0.5) and gave better results with Kariega (0.3). Cultivar Kariega was the highest contributor of genes with positive effects in SDSS (7.0) followed by Cracker (2.3) and combined to produce the highest *SCA* value (2.9), but not significant. Vitreous kernels and SKHI measures hardness of a kernel. Both traits had similar *GCA*: *SCA* ratios and were positively correlated. The lowest and highest additive genetic variances were observed for Kariega and SST 57, respectively, for both traits (Table 21). The two traits showed similarities in the SCA variances of the parents and SCA effects of the crosses. Other similar traits measured by different methods, SKWT and TGW, expressed similarity in combining abilities and variances. Parents SST 57 and Houtman had positive and significant GCA effects for P/L ratio, and SST 57 for alveograph W value.

The two locations were different in the expression of all traits (Table 18) and the genotype by environment component was predominantly GCA x E for a few traits (Table 19). The GCA x E was significant for HLW, FLY, SDSS and VK and, SCA x E for HLW and FPC. The significance of additive variance by environment interaction indicates that additive gene effects of parents were affected by environment for indicated traits. The additive genetic variances of GYD, TGW, FPC, MDT, SKHI, SKDM and SKWT were stable across environments. On the other hand, the non-additive variance by environment interaction was

observed for only HLW and FPC i.e., some specific crosses of these traits showed sensitivity to testing sites. For these traits, which *GCA* was lower than SCA or SCA was highly significant, specific combinations could be studied for better values of the traits (Baker, 1978).

Genetic and environmental causes of correlations combine to give the phenotypic correlation. If a character has high heritability, then the genetic correlation is the most important. If both characters have low heritability, the phenotypic correlation is determined by the environmental correlation (Falconer and Mackay, 1996). *GCA* and correlation between their values can give an indication about the possibility to use the means of the two parents to predict the value of the cross (Le Gouis et al., 2002). Significant negative genetic correlations were observed between GYD vs FPC (-0.9*), TKW vs VK (-0.9*), FLY vs VK (-0.9*), FLY vs SKHI (-0.9*) and SKHI vs SKWT (-0.4). Positive correlation was found between TGW vs SKWT (0.9*), FLY vs SKWT (0.7), VK vs SKHI (0.7), HLW vs SDSS (0.8), MDT vs VK (0.7), TGW vs FLY (0.7*) and GYD vs. TGW (0.9*). Grain yield alone showed negative genetic correlation with FPC, MDT, SDSS, VK, and SK- HI but significantly only with FPC. Those parameters positively correlated with grain yield (TGW, FLY, SKWT) had similar negative correlation with the same traits negatively correlated with yield. Alveograph W had significant positive phenotypic correlation with FPC (0.6**), MDT (0.6*), SDSS (0.7*), and SKHI (0.5*). The narrow sense heritability of the traits at individual and combined locations was computed from their respective variance components (Table 22).

Table 21. General and specific combining ability effect of parents and their hybrids in a combined analysis (Adet*Motta)

PN	*GYD* (g plot^{-1})	*TGW* (g)	*HLW* (kg hl^{-1})	*FLY* (%)	*FPC* (%)	*MDT* (min)	*SDSS* (ml)	*VK* (%)	SKHI	*SKDM* (mm)	*SKWT* (mg)	*P/L*	*W* (10^{-4} J)
General combining ability of the parent													
1	24.6	1.8***	0.4**	1.2***	-0.1	-0.1***	6.9***	-8.0***	-7.2***	0.1***	2.2***	-0.1	-0.5
2	-16.0	-0.9*	-0.2*	-1.0***	0.0	0.5***	0.8	7.2***	5.2***	0.0	-1.0***	0.1	3.4
3	-51.9***	-0.9*	-0.1	-0.1	0.4***	0.0	-2.5***	1.4	-0.5	-0.1***	-1.4***	-0.1	-0.9
4	31.726*	0.3	-0.6***	0.1	-0.6***	-0.2***	-7.5***	-1.7	-0.2	0.0	0.5*	0.1	-2.9
5	11.5	-0.4	0.5***	-0.1	0.1*	-0.2***	2.3***	1.0	2.7***	0.0	-0.3	0.04	0.8
SE G_i	14.2	0.4	0.1	0.2	0.1	0.0	0.7	1.0	0.7	0.0	0.3	0.02	0.4
Max mean	317.0	29.2	72.9	60.2	8.3	2.4	42.5	46.7	31.7	2.0	28.4		
Min mean	555.7	36.8	76.6	64.7	10.4	3.8	72.7	71.3	56.6	2.4	35.8		
SCA Variances of the parents													
1	19.7	1.3	0.7***	0.4	0.5***	-0.1*	4.3***	2.5	2.6*	0.1	0.7		
2	16.4	-1.0	0.8***	0.6	0.0	-0.1	-1.8	-3.7*	2.8*	0.0	-0.1		
3	-14.0	-0.8	0.4	0.2	0.3***	0.0	-0.7	4.2*	3.9***	0.0	0.3		
4	-1.7	-0.8	0.6**	0.3	0.3**	0.0	3.1*	1.9	3.2*	0.0	0.1		
5	-47.2	0.9	-0.9	-1.2	-0.9**	0.3	-3.1	-6.0	-11.0***	0.1	-1.6		
SE	28.9	0.8	0.2	0.4	0.1	0.07	1.4	2.1	1.5	0.04	0.6		
Estimates of SCA effects of the hybrids													
-SCA	-7.4(2X5)	-1.0(3X5)	-1.4(3X4)***	1.0(3X4)*	-0.4(1X3)**	-0.2(2X5)***	-5.4(1X4)	-5.0(3X4)*	-3.5(3X4)*	-0.06(1X3)	-0.7(2X4)	-3.0(2x3)	-0.1(1x2)
	-55.3(2X4)	-0.8(1X2)	-0.7(2X5)*	-0.5(2X4)	-0.4(1X4)*	-0.1(1X5)	-1.2(1X3)	-3.6(1X3)	-3.1(1X4)*	-0.03(1X2)	-0.6(1X3)	-2.2(1x4)	-0.1(2x4)
	-5.6(1X3)	-0.7(1X3)	-0.6(1X2)*	-0.4(1X5)	-0.1(2X3)	-0.1(3X4)	-0.6(1X2)	-2.5(1X4)	-2.7(2X3)	-0.02(2X4)	-0.4(1X2)	-1.9(1x2)	-0.1(3x4)
	-3.5(1X5)	-0.5(2X4)	-0.4(2X4)	-0.1(1X2)	-0.1(3X4)	-0.04(1X3)	-0.3(4X5)	-1.9(2X5)	-2.1(1X3)	-0.02(3X5)	-0.3(3X4)	-1.9(3x5)	-0.04(3x5)
	-22.2(3X4)	-0.3(1X5)	-0.2(1X3)	-0.1(1X3)	-0.1(1X2)	-0.03(4X5)		-0.1(1X2)	-1.9(1X2)	-0.01(3X4)	-0.3(3X5)	-1.2(1x3)	-0.01(1x3)
	-18.0(3X5)	-0.2(2X5)	-0.1(1X4)	-0.04(2X5)		-0.01(2X3)				-0.01(1X4)	-0.2(1X4)		-0.5(2x4)
	-13.6(1X2)											-0.5(4x5)	
+SCA	76.1(4X5)*	2.4(2X3)**	1.2(4X5)***	1.0(4X5)*	0.4(1X5)**	0.3(1X2)***	2.9(1X5)	5.6(2X4)*	4.5(1X5)*	0.04(1X5)	1.0(4X5)	-0.2(2x5)	0.1(1x5)
	59.9(2X3)	0.6(4X5)	0.9(2X3)**	0.7(3X5)	0.3(3X5)*	0.1(3X5)	1.6(2X4)	4.2(3X5)	4.3(3X5)*	0.04(2X3)	0.8(2X3)	-0.3(3x4)	0.1(4x5)
	3.1(1X4)	0.6(1X4)	0.3(3X5)	0.2(1X4)	0.1(2X5)	0.04(2X4)	1.1(3X4)	3.7(1X5)	1.9(4X5)	0.03(4X5)	0.4(1X5)		0.03(2x3)
		0.1(3X4)	0.1(1X5)	0.1(2X3)	0.1(2X4)	0.03(1X4)	0.6(2X3)	0.1(2X3)	1.63(2X4)	0.01(2X5)	0.4(2X5)	0.2(2x5)	0.02(2x5)
					0.1(4X5)		0.3(3X5)	0.0(4X5)	0.3(2X5)				
							0.6(2X5)						
SE Sij	36.6	0.9	0.3	0.5	0.2	0.09	1.8	2.7	1.8	0.05	0.8	0.07	1.0

Table 22. Narrow sense heritability estimates

	Environments			
Traits	Adet	Motta	Adet x Motta	Mean(h^2_n)
Grain yield	0.51 (0.10)	0.03 (0.03)	0.10 (0.05)	0.21
Thousand grains weight	0.18 (0.07)	0.60 (0.10)	0.22 (0.08)	0.33
Hectoliter weight	0.00*	0.14 (0.06)	0.04 (0.03)	0.06
Flour yield	0.79 (0.07)	0.29 (0.09)	0.25 (0.08)	0.46
Flour protein content	0.74 (0.07)	0.20 (0.07)	0.36 (0.10)	0.43
Mixograph development time	0.91 (0.03)	0.83 (0.06)	0.71 (0.08)	0.81
SDS Sedimentation volume	0.81 (0.06)	0.76 (0.07)	0.61 (0.10)	0.73
Vitreous kernels	0.48 (0.10)	0.72 (0.08)	0.36 (0.10)	0.52
SK-Hardness index	0.92 (0.03)	0.54 (0.10)	0.58 (0.10)	0.68
SK-seed diameter	0.57 (0.10)	0.48 (0.10)	0.29 (0.09)	0.45
SK-seed weight	0.76 (0.07)	0.52 (0.10)	0.44 (0.10)	0.57
Alveograph P/L ratio†	0.01 (0.02)			
Alveograph strength†	0.50 (0.10)			
Loaf volume†	0.00*			

*; *Negative heritability, adjusted to zero;* †, *estimated from blended samples*
( ) =numbers in brackets are standard errors (± SE) according to Becker (1985)

Grain yield and HLW were the lowest heritable traits in either single or combined environments (De La Roche, 1975; Branlard et al., 1991). Traits with moderate to high mean heritabilities were MDT (0.81), SDSS (0.73), SKHI (0.68), SKWT (0.57), VK (0.52), W (0.5), FLY (0.46) and FPC (0.43) (Orth et al., 1972; O'Brien and Ronalds, 1987; Fisher et al, 1989; Branlard et al., 1991; Barnard et al, 2002). The most stable traits with medium-high heritability at each and combined environments were MDT, SDSS, VK and SKHI. The medium to highly heritable traits (MDT, SDSS, VK, SKHI and FPC) had positive association between them while most had negative associations with yield related traits.

In this study, significant variability was observed among genotypes in all measured traits. The two locations were different and some of the characters interacted with environment, viz. HLW, FPC, SDSS, and VK. The *GCA* was predominant over *SCA* for most traits in separate and combined environments i.e. *GCA*: *SCA* ranging from 0.7 to 37. Many authors (Morgan et al., 1989; Borghi et al., 1989; Labuschagne et al., 1996; Perenzin et al., 1998; Le Gouis et al., 2002) have reported the greater contribution of additive over non-additive genetic variance for many parameters in wheat. Therefore, the non-significant *SCA* effects of most characters permit the prediction of performance of these traits in progeny based on *GCA* alone (Baker, 1978). Generally, the presence of significant dominance gene effects and interaction of additive and non-additive variances with environment in some traits could be compensated by the preponderance of additive gene effects. It was explained with small margin of mean

differences between hybrids/crosses for those traits showing environmental sensitivity. Mixograph development time was entirely independent of environment and has no interactions. On the other hand, transgressive segregates could be expected from those crosses and traits showing significant positive non-additive gene effects i.e. HLW, FPC, and SDSS. Specific parent combinations with high *SCA* values for some traits could be exploited (Perenzin et al., 1998).

There were significant positive genetic correlations between GYD, TGW and FLY and negative correlations between GYD vs FPC, FLY vs VK/SKHI, SKWT vs SKHI. The correlations of FPC with MDT and SDSS were not significant but positive in this study. The absence of strong relationship between protein and mixing time (Khan et al., 1989) should not be surprising since mixing characteristics would be dependent on protein composition and quality. The negative relationship between grain yield and protein was obtained in high yielding crosses (Houtman x Cracker and Kariega x Houtman). However, simultaneous increase was observed for some specific combinations (Kariega x SST825, Kariega x SST57) of high yielding and high protein parents. Therefore, selection of appropriate parents for crossing to get high yield and protein lines could be possible (Costa and Kronstad, 1994; Fabrizius et al., 1997). Kariega, SST57 and Cracker were good combiners i.e., Cracker for HLW, FPC, SDSS and SKHI; Kariega for TGW, HLW, FLY, SDSS, SKDM and SKWT; SST57 for MDT, VK, SKHI). In addition, Kariega is also a good specific combiner for FPC, SDSS and SKHI i.e., SCA was significant for these traits. Hybrid Kariega x Cracker showed similar increment in *SCA* effects for protein (0.4), SDSS (2.9), SKHI (4.5) but the two parents had variable GCA effects for these traits.

Heritability of a character can be classified as low, 5-10 %, medium, 10-30 %, and high, 30-60 % (Dabholkar, 1992). High heritability estimates were found for most traits in the high yielding environment and a similar class trend was observed for some traits in the low yielding environment i.e. fluctuation of heritability was low for some traits. The heritability of MDT, SDSS, VK, SKHI and SKWT was high in these sets of crosses and environments. Grain yield, TGW and HLW had variable heritabilities. Therefore, selection using highly heritable traits is desirable for selection gains. In general, characters that are controlled by genes in additive fashion have higher values of heritability than the characters governed by genes with large non-additive effects (Dabholkar, 1992).

The range of positive heterosis was 0-11 % for most traits studied. There was no positive heterosis for SDSS, MDT, SKHI and SKDM in these combinations. The highest positive heterosis was achieved by the crosses Houtman x Cracker in GYD (11.9 %) and SST 57 x SST 825 in TGW (10.9 %), the later cross also showing positive heterosis in HLW (0.4%) and SKWT (2.1 %). The cross SST 57 x Cracker showed little advantages in FLY (0.6 %), TGW (1.3 %) and SKWT (0.7 %).

5.5 Conclusion

In this study with these specific parents, utilizing the additive genetic variances would be effective to improve the quality traits i.e., MDT, SDSS, FLY, VK and TGW, the same traits had medium to high heritabilites, and phenotypic selection could be effective. The similarities of mean rankings (MDT, FPC, and SDSS), positive rankings of GCA effects (FPC, MDT, SDSS, VK, SKHI and SKWT), heritabilities and correlations for important quality traits across both locations will facilitate the use of any one of these locations for selection for both locations. The protein level was generally low due to the influence of diseases. However, the amount of nitrogen applied was higher to secure a better level of protein. Further research into the management and environmental factors to enhance protein content is necessary.

5.6 References

AACC (American Association of Cereal Chemists), 2000. Approved methods of the AACC. 10th edition. The association: St. Paul, MN.

Baker, R.J. and Sutherland, K.A., 1991. Inheritance of kernel hardness in five spring wheat crosses. *Can. J. Plant Sci.* 71:179-181.

Baker, R.J., 1978. Issues in diallel analysis. *Crop Sci.* 18:533-536.

Barnard, A.D., Labuschagne, M.T. and van Niekerk, H.A., 2002. Heritability estimates of bread wheat quality traits in Western Cape province of South Africa. Euphytica 127:115-122.

Beck, D.L., Vasal S.K., and Crossa J., 1991. Heterosis and combining ability among subtropical and temperate intermediate-maturity maize germplasm. *Crop Sci.* 31:68-73.

Becker, W.A., 1985. Manual of quantitative genetics. Fourth edition. McNaughton and Gunn, Inc. Ann Arbor, Michingan, USA. *p.*48-52.

Bingham, J. and Lupton, F.G.H., 1987. Production of new varieties: an integrated research approach to plant breeding. *In*: Lupton, F.G.H.(*Ed.*). Wheat breeding, its scientific basis. Chapman and Hall, London and New York. *pp.*487-538.

Bitzer, M.J., and Fu, S.H., 1972. Heterosis and combining ability in southern soft red winter wheat. *Crop Sci.*12: 35-37.

Blackman, J.A., and Payne, P.I., 1987. Grain quality. *In:* Lupton, F.G.H *(Ed).* Wheat breeding, its scientific basis. Chapman and Hall, London and New York. *pp.455-485.*

Borghi, B., Corbeellini, M., and Perenzin, M., 1987. Bread making quality of F_1 hybrids produced using chemical hybridizing agent. *In:* Borghi, B. *(Ed.).* Hard wheat: Agronomic, technological, biochemical and genetic aspects. Commission of the European communities, Luxembourg. *pp. 225-236.*

Borghi, B., Perenzin M., and Nash, R.J., 1989. Combining ability estimates in bread wheat and performance of 100 F_1 hybrids produced using a chemical hybridizing agent. *J. Genet. Breed.* 43:11-16.

Branlard, G., Rousset, M., Loisel, W. and Autran, J.C., 1991. Comparison of 46 technological parameters used in breeding for bread wheat quality evaluation. *J. Genet & Breed.* 45:263-280.

Brears, J., and Bingham J., 1989. Exploitation of heterosis in hybrid wheat using gametocytes. *Vorträge fur pflanzenzüchtüng* 16:397-409.

Costa, J.M., and Kronstad, W.E., 1994. Association of grain protein concentration and selected traits in hard red winter wheat populations in the Pacific Northwest. *Crop Sci.* 34:1234-1239.

Dabholkar, A.R., 1992. Elements of biometrical genetics. Ashok Kumar, Mittal Ltd., New Delhi, 431 pp.

Dhillon, B.S., Paul, Chr., Zimmer, E., Gurrath, P.A., Klein, D., and Pollmer, W.G., 1990. Variation and covariation in stover digestibility traits in diallel crosses of maize. *Crop Sci.* 30:931-936.

Edwards, I.B., 1987a. Breeding of hybrid varieties of wheat. *In:* hybrid seed production of selected cereals, oil and vegetable crops. FAO Plant protection and production paper 82, FAO, Rome. *pp.* 3-25.

Edwards, I.B., 1987b. Baking quality of hard red winter and hard red spring wheat hybrids. *In:* Borghi, B. *(Ed.).* Hard wheat: Agronomic, technological, biochemical and genetic aspects. Commission of the European communities, Luxembourg. *pp.* 215-224.

Fabrizius, M.A., Cooper, M. and Basford, K.E., 1997. Genetic analysis of variation for grain yield and protein concentration in two wheat crosses. *Aust. J. Agric. Res.* 48:605-614.

Falconer, D.S. and Mackay, T.F.C., 1996. Introduction to quantitative genetics (Fourth edition). The Longman Press, London. 464 *pp.*

Fisher, R.A., O'Brien, L. and Quail, K.J., 1989. Early generation selection in wheat. II. Grain quality. *Aus. J. Agric. Res. 40:1117-1133.*

Fowler, D.B. and De La Roche, I.A., 1975. Wheat quality evaluation. 2. Relationship among prediction tests. *Can. J. Plant Sci.* 55:251-262.

Griffing, B., 1956a. Generalized treatment of the use of diallel crosses in quantitative inheritance. *Heredity* 10:31-50.

Griffing, B., 1956b. Concepts of general and specific combining ability in relation to diallel crossing systems. *Aus. J. Biol. Sci.* 9:463-493.

Khan, K., Frohberg, R., Olsen, T. and Huckle, L., 1989. Inheritance of gluten protein components of high-protein hard red spring wheat lines derived from *Triticum turgidum* var *diccoides*. *Cereal Chem.* 66(5):397-401.

Labuschagne, M.T., Coetzee, M.C.B. and van Deventer, C.S., 1996. General combining ability of six genotypes of spring wheat (*Triticum aestivum* L.) for biscuit making quality characteristics. *Plant Breed.* 115:279-281.

Le Gouis, J., Beghin, D., Heumez, E., and Pluchard, P., 2002. Diallel analysis of winter wheat at two nitrogen levels. *Crop Sci.* 42:1129-1134.

Matuz, J., Kertész Z. and Ács, E., 1993. Inheritance of bread making quality in crosses of Hungarian and North-American winter wheats (*Triticum aestiveum* L.). *Cereal. Res. Commun.* 21(1): 39-43.

Morgan, C.L., Austin, R.B., Ford, M.A., Bingham, J., Angus, W.J., and Chowdhury, S., 1989. An evaluation of F_1 hybrid winter wheat genotypes produced using a chemical hybridizing agent. *J. Agri. Sci. Camb.* 112: 143-149.

Morgan, C.L., 1998. Mid-parent advantage and heterosis in F_1 hybrids of wheat from crosses among old and modern varieties. *J. Agri. Sci. Camb.*130:287-295.

O'Brien, L., and Ronalds, J.A., 1987. Heritabilities of small scale and standard measures of wheat quality for early generation selection. *Aust. J. Agric. Res.* 38:801-808.

Orth, R.A., Baker, R.J and Bushuk, W., 1972. Statistical evaluation of techniques for predicting baking quality of wheat cultivars. *Can. J. Plant Sci.* 52: 139-146.

Perenzin, M., Corbellini, M., and Borghi, B., 1987. Growth analysis of ten bread wheat *hybrids (T. aestivum)* produced with a chemical hybridizing Agent. *Genetica Agraria* 41: 163-172.

Perenzin, M., Corbellini, M., Accerbi, M., Vaccino, P. and Borghi. B., 1998. Bread wheat: F_1 hybrid performance and parental diversity estimates using molecular markers. *In*: Braun H.J. et al. *(Eds.).* Wheat: Prospects for global improvement. *pp.339-345.*

Pfeffer, A., and Zeller, F.J., 1987. Genotypes of hybrid necrosis in 25 spring varieties of common wheat (*Triticum aestivum* L.). *Plant Breed.* 99:83-84.

Pickett, A.A., 1993. Hybrid wheat results and problems. Advances in plant breeding. Supplement 15 to plant breeding. Scientific Publishers, Berlin and Hamburg.

Rodriguez, R., Quinones, M.A., Borlaug, N.E. and Narvaez, I., 1967. Hybrid wheats: Their development and food potential. Research Bulletin 3. International Maize and Wheat Improvement Centre, Londres, Mexico.

Stuber, C.W., Johnson, V.A. and Schmidt, J.W., 1962. Grain protein content and its relation ship to other plant and seed characters in the parent and progeny of a cross of *Triticum aestivum* L. *Crop Sci.*2: 506-508.

Sughroue, J.R., and Hallauer, A.R., 1997. Analysis of the diallel mating design for maize inbred lines. *Crop Sci.* 37:400-405.

Zhang, Y., and. Kang, M.S., 1997. DIALLEL-SAS: A SAS program for Griffing's diallel analysis. *Agron. J.* 89:176-182.

Chapter 6 Variability for quality traits and bread making in Ethiopian durum wheat cultivars and advanced lines

6.1 Abstract

In Ethiopia, durum wheat is mainly used for local bread making and other food types as well as pasta products. Fifteen advanced lines including three commercial cultivars were sampled and tested at two environments, Adet and Motta, in northwestern Ethiopia. Grain yield and thirteen quality traits were investigated. The mean flour protein content (FPC) of genotypes ranged from 10.1-12.5 % and 6.7-8.1 % at Adet and Motta, respectively. The SDS-sedimentation (SDSS), hardness index (SKHI) and vitreousness (VK) values were reduced and the mixing time (MDT) increased at lower protein environment. The mean MDT of all genotypes was 4 min at Motta and 2.8 min at Adet and SDSS ranged between 10.7-32.3 ml across both locations. In a combined analysis, there were significant genotypic differences only for FPC and VK. The large non-significant genotypic difference for the rest of the other traits could be due to larger environmental variation. The repeatability was high for flour yield, FPC, VK and SKHI. Flour protein content was negatively correlated with MDT but highly positively correlated with VK and SKHI at both environments. The bread loaf volume ranged from 483-730 cm^3 at protein levels between 10.1-12.5%. Bread volume had strong positive correlation with SDSS but was weakly associated with FPC. Higher loaf volumes could be achieved by selection for higher SDSS and FPC genotypes. Stepwise regression was used to select variables explaining the variability of MDT. Flour protein content, SDSS and seed weight were selected and used in multiple regression analysis to establish the prediction model and which explained 69 % of the variation for MDT. This preliminary study in the region indicated the need to study management aspects for higher protein, establish quality criteria for selection and incorporation in the current breeding strategy to help farmers grow good-quality wheat for domestic markets.

Key words: Durum wheat, Ethiopian advanced lines, prediction models, quality traits

6.2 Introduction

Durum wheat (*Triticum turgidum* L. (Thell.) *ssp. Durum* (Desf.) Husn.) is the second most cultivated wheat species in the world, next to common wheat, with 27 million tons of

production (Peña et al., 2002). It is generally milled into semolina (coarse grits) and is widely used to produce superior pasta products and regionally in the production of flat breads and other important foods. Ethiopia is considered as a center of genetic diversity for durum wheat species (Vavilov, 1931; 1951), and possesses huge diversity for genetic improvement (Tessema, 1988; Demissie et al., 1990) and thousands of accessions are collected and preserved. The Ethiopian durum wheat accessions have shown the highest diversity index (Negassa, 1986; Jain et al., 1975) and are important sources for rust resistance, long coleoptiles, short culms, low tillering, early ripening, drought resistance and waterlogging tolerance (Porceddu, 1976; Payne et al., 2001). A protein content of 22 % for some accessions was recorded (Demissie et al, 1990). Most of the studies indicated the presence of genetic diversity in many agronomic traits of indigenous materials for possible genetic improvement (Tessema, 1988; Demissie et al., 1990) and the breeding effort should be strengthened to exploit the expected potential. The durum wheat breeding program of Ethiopa emphasized improvement of the yielding ability of landraces (Tessema, 1987).

Durum wheat lacks the gluten strength found in most bread wheats (*T.aestivum*) due to the absence of the D-genome. Chromosome 1D carries major determinants of gluten strength and baking quality (Schmidt et al., 1966; Morris et al., 1968). However, improvement is possible through breeding, as substantial variability exists for gluten strength and baking performance among durum wheats (Boggini and Pogna, 1989; Peña et al., 1994). In Ethiopia, durum wheat is consumed largely in the form of whole-wheat fermented/leavened local bread (Dabo, dense or raised) or as flat breads and other different forms of food such as roasted grain (Kolo), boiled whole grain with beans or alone (nifro), and pan cake type bread (Injera). Traditional fermented bread making process from whole-wheat flour is similar for both durum and bread wheat. Most of the produce is consumed in the form of local fermented bread. There is a great need to strengthen breeding for nutritive and processing quality (Bechere et al., 2001) to satisfy the increasing demands. Although the country is the origin and/or diversity of durum wheat, some durum wheat processing industries depend on the importation of raw materials or are unable to utilize available local produce. This may be due to easily resolvable/researchable constraints i.e., the domestic supply of raw material is negligible (Dessalegn, 2001, Survey information supplementary to this study, unpublished). Some cultivars released for agronomic superiority offer variability and good processing quality (Bechere et al., 2001). Northwestern Ethiopia, which is among the major durum wheat growing regions, has endogenous diversity and the potential for production and supply of raw materials. The aim of this study was to

investigate the variability for grain yield and quality in some Ethiopian durum wheat cultivars and advanced lines tested in northwestern Ethiopia and study the association between the quality parameters, examine the causes of variations and correlations.

6.3 Material and methods

Fifteen genotypes including advanced lines with potential for release and three popularly grown commercial cultivars were sampled. They were all originally bred for their agronomic superiority with no firm selection for quality. The materials were grown at two environments in northwestern Ethiopia viz., Adet research center and Motta, in a randomized complete block design with three replications. The plot size was 3 m^2 consisting of six rows, 20 cm apart and, 2.5 m length. Standard agronomic practices were followed. After harvest, grain yield and quality parameters were measured (Table 23). Seeds of the three replications of each cultivar/line were blended, cleaned, well mixed and a sample was taken to determine bread volume. The normality was checked for values within the expected ranges using the Omnibus test based on skewness and kurtosis (D'Agostino et al., 1990). The analysis of variance was computed and the estimates of variances were obtained from the expectation of mean squares (Singh et al, 1993) and/or using RELM of SAS (SAS, 1994) as: single environment, genotypic variance $(\sigma^2_g) = \frac{(Mg - Me)}{b}$; More environments, $\sigma^2_g = \frac{(Mg - Mi)}{bl}$; genotype by environment interaction variance $(\sigma^2_i) = \frac{(Mi - Me)}{b}$; error variance $(\sigma^2_e) = Me$; b, block; l, environment; Mg, mean square of genotype; Mi, mean square due to interaction; Me, error mean square. Phenotypic correlations (Spearmans' rank) were calculated using NCSS software (NCSS, 2001) for each and combined environments. Repeatability (b_i) of the traits was calculated from their variance components (Becker, 1984; Singh et al., 1993) as: single environment, $b_i = \frac{\sigma^2_g}{\sigma^2_g + \sigma^2_e}$; more environments, $b_i = \frac{\sigma^2_g}{\sigma^2_g + \sigma^2_i + \sigma^2_e}$. The stepwise regression procedure was used to select the best independent quality variables, which describe the selected dependent variable. The selected independent variables were used in multiple regressions to establish the prediction equation (NCSS, 2001) using the linear model: $Y = b_0 + \beta_1 X_1 + \beta 2X2 + \beta_3 X_3 + \ldots\ldots + \beta_n X_n$; b_0, the intercept; $\beta_{1\text{-}n}$, the slopes of the regression plane; $X_{1\text{-}n}$, independent variables; Y, dependent variable. Coefficient of determination (R^2) was calculated to indicate the percent of variation

in the dependent variable explained by selected independent variables in the model: $R^2 = \frac{SS_{model}}{SS_{total}} . R^2_{Predict}$, which reflects the prediction ability of the model, was calculated as:

$R^2_{predict} = 1 - \frac{PRESS}{SS_{Total}}$ where $PRESS = \sum (y_i - \hat{y}_{i,-i})^2$; $R^2_{predict}$ must be closer to R^2.

Table 23. Methods, units and abbreviations of measurements of quality traits

Traits	Abbreviations	Unit	Methods of measurements
Grain Yield	GYD	ton ha^{-1}	
1000 grains weight	TGW	g	
Hectoliter weight	HLW	kg hl^{-1}	
Break flour yield	BFLY	%	AACC 26-21A
Flour yield	FLY	%	AACC 26-21A† and Junior Quadromat mill
Flour protein content	FPC	%	AACC 39-11
Falling number	FLN	sec	AACC 56-81B
SDS-sedimentation test	SDSS	ml	AACC 56-70
Vitreous kernels	VK	%	Sliced/counted
SKCS- hardness index	SKHI		AACC 53-31
SKCS-seed diameter	SKDM	mm	AACC 53-31
SKCS-seed weight	SKWT	mg	AACC 53-31
Mixograph development time	MDT	min	AACC 54-40A
Loaf volume	LFV	cm^3	AACC 10-09

SKCS, Single Kernel Characteristics System; †, Bühler method for only blended samples; AACC, American Association of Cereal Chemists (2000)

6.4 Results and discussion

Fourteen grain yield and quality traits were investigated (Table 23) at two environments. At Motta, the mean protein content of the genotypes was very low (6.7 to 8.1 %). This resulted in overall longer MDT, lower SDSS volume and lower vitreousness or hardness index (Table 24). The average mixing time of all varieties was 4 min. SDSS ranged from 10.7 to 27.7 ml (mean of 19.9 ml). The strongest lines were CD96486 (MDT of 5.3 min), DZ1652 (5.3 min) and DZ900 (4.7 min). They also had the highest SDSS volume. DZ1640 had the lowest vitreousness (and hardness index) and protein content but had the highest value of MDT and SDSS. On the other hand, DZ1748 had the highest vitreousness, FPC and SDSS with relatively shorter mixing time. Therefore, it showed that there should be an optimum balance between values of these parameters. The yield of all genotypes was very low (1.9-2.7 ton ha^{-1}) as opposed to the potential of the area (Dessalegn and Wondale, 1998). The leading cultivar in

yield, DZ1748, had the highest TGW, HLW, SKDM and SKWT. This was caused by waterlogging which retarded overall performance. Most of the genotypes had 1000 grain weights greater than 35g. The hectoliter weight and flour yield showed less variability and ranged between 75.3-77.8 kg hl^{-1} and 56.4-59.6 %, respectively. The flour yield (Quadromat flour mill) was higher at the lowest yielding site. Generally, higher mean flour yields were obtained from higher yielding cultivars/lines.

Table 24. Mean yield and quality traits of genotypes grown at Motta

NAME	GY	TGW	HLW	FLY	FPC	MDT	SDSS	VK	SKHI	SKDM	SKWT
CD 96486	1.9	36.7	75.9	60.1	7.2	5.3	21.3	43.3	47.2	2.5	34.2
CD 6630	1.3	38.0	77.5	60.8	8.1	3.4	19.3	62.7	56.4	2.6	37.1
CD 95294-1Y	1.7	37.2	76.7	60.9	7.2	4.1	20.0	47.3	48.9	2.4	34.4
DZ 2023	1.5	36.0	76.8	63.8	7.2	3.9	19.7	36.0	41.2	2.6	36.6
YILMA	1.3	36.6	76.4	62.4	7.4	4.7	27.7	54.0	52.1	2.5	36.1
DZ 1721	1.4	36.7	74.3	61.9	7.5	2.6	10.7	48.7	48.0	2.6	36.2
DZ 2212	1.1	35.8	79.2	60.0	7.6	3.6	20.3	58.7	49.4	2.5	35.7
DZ 1924	1.1	36.2	76.6	61.9	7.4	3.0	15.0	54.0	52.3	2.5	35.8
BICHENA	1.3	36.4	74.0	61.8	7.2	4.1	21.7	51.3	43.4	2.5	36.5
LD 357	1.3	31.2	75.6	61.8	7.1	3.7	15.0	38.0	45.4	2.3	30.6
DZ 1640	1.9	36.1	75.9	62.7	6.7	4.8	21.0	28.0	35.0	2.4	33.9
DZ 1691	1.5	37.3	77.2	61.2	7.8	3.1	18.3	44.7	50.4	2.5	36.2
DZ 1652	1.4	33.1	76.6	59.9	7.4	5.3	23.7	43.3	45.7	2.3	32.2
DZ 1748	1.9	40.1	77.1	60.5	8.1	3.7	23.7	64.7	55.5	2.8	40.4
DZ 900	1.9	35.8	76.4	61.2	7.0	4.7	21.0	35.3	50.1	2.4	32.7
Grand mean	1.5	36.2	76.4	61.4	7.4	4.0	19.9	47.3	48.1	2.5	35.3
CV (%)	26.5	9.1	1.7	3.4	8.3	36.5	32.4	31.2	16.5	6.8	8.5
LSD (0.05)	0.6	4.6	1.8	2.9	0.9	2.0	9.0	20.5	11.0	0.2	4.1

On the other hand, at Adet, cultivars/lines scored higher protein contents of up to 14 % (plot basis) and the mean protein content ranged from10.1-12.5 %. They had relatively shorter mixing time, higher SDSS and vitreousness (hardness index) than at Motta (Table 25). Lines with the strongest gluten strength were DZ 1721, CD96486, DZ1924 and CD6630 i.e., with longer MDT and higher SDSS values. The same lines had a higher percentage of vitreousness and hardness index with above average loaf volume. DZ 2212, CD 6630 and DZ 1748 obtained the highest mean number of vitreous kernels and similar lines had the highest SKHI. Generally, the mean SKWT, TGW, MDT and SKHI were higher than for the bread wheat genotypes grown simultaneously as durum wheat is known to have the hardest, most vitreous and heaviest grain of all wheats (Liu et al., 1996). Buhler flour yields were higher and gave more precise results than Quadromat milling yield since the method uses larger samples (Simmonds, 1989).

Table 25. Mean of yield and quality traits of genotypes grown at Adet

NAME	GYD	TGW	HLW	FLY	FPC	MDT	SDSS	VK	SKHI	SKDM	SKWT	LFV	BFLY	FLY^2	FPC^2
CD 96486	2.7	33.4	77.2	54.6	10.8	3.3	32.3	76.0	63.4	2.4	34.5	730	14.6	67.7	11.5
CD 6630	2.5	36.1	74.4	52.5	12.5	2.8	24.3	89.3	69.6	2.5	36.0	678	14.4	68.7	11.3
CD 95294-1Y	3.2	38.2	77.3	54.1	11.4	3.0	23.3	87.3	71.2	2.6	37.3	615	13.1	66	11.0
DZ 2023	3.6	38.4	75.3	55.3	10.0	2.7	24.3	80.7	65.5	2.6	37.9	683	16	70.5	10.8
YILMA	2.9	37.4	75.2	54.8	12.5	2.7	22.7	85.3	72.0	2.5	35.9	561	13	66	11.1
DZ 1721	3.3	36.5	76.8	54.0	10.7	3.8	26.7	80.7	67.1	2.5	36.2	672	14.2	67.8	10.2
DZ 2212	2.9	38.8	76.4	53.4	11.9	2.4	24.3	97.3	71.1	2.6	37.2	606	12.7	66.4	11.1
DZ 1924	3.2	38.6	77.7	54.0	11.9	3.0	26.7	90.7	71.1	2.6	36.9	661	12.5	66.2	11.1
BICHENA	3.4	40.5	76.5	54.6	10.5	2.7	23.0	87.3	66.5	2.8	41.7	679	13.7	66.9	9.9
LD 357	3.4	36.7	75.5	56.1	10.1	2.7	18.0	66.7	63.7	2.6	36.8	555	19.3	72.3	10.0
DZ 1640	3.5	37.4	76.1	56.0	10.1	2.8	19.7	78.7	62.9	2.6	35.9	549	14.2	67.3	10.4
DZ 1691	2.7	36.6	76.5	55.9	11.4	2.6	23.7	84.7	67.5	2.6	35.5	567	14.1	67.5	11.2
DZ 1652	2.7	33.8	76.4	52.7	11.2	2.6	20.7	90.0	68.7	2.5	36.2	621	13	67.2	11.1
DZ 1748	2.9	36.0	75.9	55.5	10.9	2.7	28.0	86.7	67.1	2.5	35.0	671	14.2	67.5	10.1
DZ 900	3.5	40.6	76.1	55.4	11.0	2.3	18.3	73.3	67.2	2.6	38.2	483	16.5	69.8	10.8
Grand Mean	3.1	37.3	76.2	54.6	11.1	2.8	23.7	83.6	67.6	2.6	36.7	621.1	14.4	67.9	10.8
CV (%)	19	14.1	2.6	3.1	10.9	29.3	36.4	14.9	9.1	10.6	13.5				
LSD(0.05)	0.8	7.3	2.8	2.4	1.7	1.1	12.0	17.3	8.6	0.4	6.9				

LFV, BFLY, FLY^2 and FPC^2 were determined from blended samples; BFLY and FLY^2, from Bühler flour extraction method

Loaf volumes were determined from blended seed obtained from the highest protein site, Adet. Higher bread loaf volume (661-730 cm^3) was achieved from genotypes with a mixing time of 2.7-3.8 min, SDSS of 24.3-32.3 ml, and protein levels of above 10 % (Table 24). Advanced line CD 96486 scored the highest loaf volume (730 cm^3) having the highest SDSS (32.3 ml) and a mixing time of 3.3 min. A high SDSS volume was associated with large loaf volume (Figure 12). The lowest loaf volume (483 cm^3) was achieved by DZ900 with the lowest SDSS and MDT values.

The average mixing time of all genotypes at both locations was high: 4 min at Motta, 2.8 at Adet and 3.4 min when combined (Table 26). Cultivars such as CD95294-1Y, CD96486, LD357 and DZ900 had the longest mixing time (plot basis) of more than 6 min at the lowest protein site. The mean SDSS sedimentation of lines/cultivars was generally lower (10.7-32.3 ml) across both locations. Lower SDSS is an indication of poorer gluten strength in durum wheats (Finney et al., 1987) and was also affected by the level of protein content of the genotypes. Cultivars/lines grown at the highest protein environment, Adet, expressed shorter mixing time, higher SDSS volume, higher vitreousness percentage and hardness index than the lowest protein environment, Motta. The alpha-amylase activity, an important grading factor, was very low for all genotypes at both locations with a falling number greater than 410 sec.

In a combined analysis, there was a significant difference between the two sites i.e., the environmental variation was high as the environment plays an imoportant role in the expression of many quality traits (Rharrabti et al., 2003). The tested genotypes showed differences only in FPC and VK. The non-significant differences between genotypes in most traits could be due their closer genetic backgrounds, lower genetic variances and high CV values (Table 26 and 27). Negative variances were obtained for genotypes and genotype by environment interaction variances for some traits which can be caused by sampling variation and the trials must be repeated over seasons and years to overcome the probability of negative variances (Dudley and Moll, 1969). The repeatability of the traits was higher for FLY, FPC, VK and SKHI in the Adet x Motta combination. Negative repeatabilities, which were caused by negative variances, were adjusted to zero.

Table 26. Mean of cultivars/lines for yield and quality traits studied across environments

NAME	GYD	TGW	HLW	FLY	FPC	MDT	SDSS	VK	SKHI	SKDM	SKWT
CD 96486	2.3	35.1	76.5	57.3	9.1	4.3	26.8	59.7	55.3	2.48	34.4
CD 6630	1.9	37.1	75.9	56.6	10.3	3.1	21.8	76.0	63.0	2.57	36.6
CD 95294-1Y	2.5	37.7	77.0	57.5	9.3	3.6	21.7	67.3	60.1	2.55	35.8
DZ 2023	2.5	37.2	76.1	59.6	8.6	3.3	22.0	58.3	53.3	2.60	37.3
YILMA	2.1	37.0	75.8	58.6	9.9	3.7	25.2	69.7	62.1	2.55	36.0
DZ 1721	2.4	36.6	75.5	58.0	9.1	3.2	18.7	64.7	57.6	2.57	36.2
DZ 2212	2.0	37.3	77.8	56.7	9.8	3.0	22.3	78.0	60.2	2.54	36.5
DZ 1924	2.1	37.4	77.2	58.0	9.7	3.0	20.8	72.3	61.7	2.57	36.3
BICHENA	2.4	38.5	75.3	58.2	8.9	3.4	22.3	69.3	55.0	2.67	39.1
LD 357	2.4	34.0	75.5	59.0	8.6	3.2	16.5	52.3	54.5	2.45	33.7
DZ 1640	2.6	36.8	76.0	59.3	8.4	3.8	20.3	53.3	48.9	2.48	34.9
DZ 1691	2.1	36.9	76.9	58.5	9.6	2.9	21.0	64.7	59.0	2.55	35.9
DZ 1652	2.1	33.5	76.5	56.4	9.3	4.0	22.2	66.7	57.2	2.43	34.2
DZ 1748	2.4	38.0	76.5	58.0	9.5	3.2	25.8	75.7	61.3	2.63	37.7
DZ 900	2.7	38.2	76.3	58.3	9.0	3.5	19.7	54.3	58.7	2.55	35.5
Grand mean	2.3	36.7	76.3	58.0	9.3	3.4	21.8	65.7	57.8	2.5	36
CV (%)	21.8	11.9	2.2	3.3	10.4	34.9	35.0	20.9	12.3	8.9	11.4
LSD(0.05)	0.5	4.2	1.6	1.8	0.9	1.1	7.4	13.2	6.9	0.2	4
R^2	0.83	0.26	0.39	0.87	0.87	0.47	0.37	0.78	0.79	0.32	0.33
Adet, Range	2.5-3.6	33.4-40.6	74.4-77.7	52.5-56.1	10.0-12.5	2.3-3.8	18.0-32.3	66.7-97.3	62.9-72.0	2.4-2.8	34.5-41.7
Motta, Range	1.1-1.9	31.2-40.1	74.0-79.2	59.9-63.8	6.7-8.1	2.6-5.3	10.7-27.7	28.0-64.7	35.0-56.4	2.3-2.8	30.6-40.4
Combined	1.9 - 2.7	33.5-38.5	75.3-77.8	56.4-59.6	8.4-10.3	2.9-4.3	16.5-26.8	52.3-78.0	48.9-63.0	2.4-2.7	33.7-39.1

The falling number was more than 410 sec for all cultivars and lines; DF of error =56; Range, mean basis

Table 27. Analysis of variance, variance components and repeatability of quality traits

	Mean square				Variance estimates				Repeatability	
Source	Env	Gen	Gen X Env	Error	Gen	Gen X Env	Error	Adet X Motta	Adet	Motta
GY	57.50***	0.40	0.28	0.27	0.01	0.03	0.29	0.08 (0.07)	0.25 (0.17)	0.33 (0.17
TGW	24.90	13.00	11.70	19.20	0.22	-2.50	19.20	0.10 (0.07)	-1.17 (0.45)	0.09 (0.17
HLW	0.60	2.90	4.20	2.80	-0.22	0.45	2.80	-0.45 (0.17)	-0.74 (0.13)	0.64 (0.13
FLY	1045.20***	5.50	1.90	3.60***	0.60	-0.57	3.60	0.65 (0.10)	0.26 (0.17)	-0.23 (0.10
FPC	314.30***	1.80*	0.70	0.90***	0.18	-0.07	0.90	0.61 (0.10)	0.25 (0.17)	0.20 (0.17
MDT	32.30***	1.00	1.50	1.40	-0.08	0.03	1.40	-0.50 (0.20)	-0.75 (0.14)	-0.02 (0.15
SDSS	332.50*	42.80	49.90	58.10	-1.18	-2.73	58.10	-0.17 (0.02)	-0.74 (0.13)	0.17 (0.17
VK	29666.20**	422.70**	89.10	186.7***	55.60	-32.53	186.70	0.79 (0.07)	0.14 (0.17)	0.34 (0.17
SKHI	8619.60***	89.70	27.60	50.6***	10.35	-7.67	50.6	0.69 (0.09)	-0.43 (0.03)	0.30 (0.17
SKDM	0.10	0.02	0.04	0.05	-0.003	-0.003	0.05	-1.00 (0.62)	-2.50 (2.16)	0.40 (0.17
SKWT	50.00	11.90	13.60	16.8	-0.28	-1.07	16.8	-0.14 (0.01)	-1.78 (1.10)	0.46 (0.16

, **, * indicates significant at P<0.05, P<0.01 and P<0.001, respectively; Env, environment; Df, degrees of freedom; Gen, genotype; Df for environment, 1; Genotype by environment, 14; error, 56;*

( ), numbers in brackets are standard errors (±SE) according to Becker (1985)

The correlations between grain yield and quality traits varied at low and high levels of protein environments. Thousand grains weight was strongly and positively correlated with SKWT and SKDM, moderately to SKHI and weakly and negatively with MDT and SDSS (Table 28). The relationship of vitreousness (SKHI or VK) to HLW and TGW was stronger at the lower protein site, Motta. Similarly, FPC was positively related with TGW and HLW. Flour yield was negatively correlated with FPC, VK and SKHI i.e., the harder grains produced less flour due to less starchy kernels. Flour protein content was negatively correlated with MDT but its negative association weakened when the protein levels increased. It was also strongly and positively correlated with VK and SKHI at all environments, single or combined. Hard wheat had higher percentages of vitreousness (less yellow berry) related to higher protein contents. Mixing time had positive correlation with SDSS i.e., strong grains with higher flour protein content resulted in higher SDSS volume. Vitreous kernel percentage and SKHI were highly correlated as they measure the same parameter. Similarly, SKWT and SKDM were also highly correlated, as larger seeds will have higher weights and diameter. Flour yield was strongly related to BFLY (0.91). BFLY was negatively and weakly correlated with vitreousness. Loaf volume did not show a strong relationship with FPC or VK but was weakly associated with SDSS volume.

Table 28. Phenotypic correlation of the studied quality traits

		GY	TGW	HLW	FLY	FPC	MDT	SDSS	VK	SKHI	SKDM	VK^2	BFLY	FLY^2	FPC^2
TGW	Adet	0.65*													
	Motta	0.25													
	combined	0.30*													
HLW	Adet	-0.06	0.07												
	Motta	-0.10	0.27												
	combined	0.05	0.00												
FLY	Adet	0.48	0.09	-0.24											
	Motta	-0.05	-0.11	-0.40											
	combined	-0.61***	-0.16	0.01											
FPC	Adet	-0.68*	-0.01	0.03	-0.54*										
	Motta	-0.32	0.58*	0.62*	-0.40										
	combined	0.61***	0.22*	0.14	-0.78***										
MDT	Adet	-0.02	-0.33	0.38	-0.20	-0.11									
	Motta	0.48	-0.31	-0.30	-0.15	-0.59*									
	combined	-0.35***	-0.28*	0.05	0.34**	-0.46***									
SDSS	Adet	-0.38	-0.34	0.33	-0.37	0.15	0.49								
	Motta	0.29	0.00	-0.04	-0.28	-0.07	0.76**								
	combined	0.22*	-0.05	0.10	-0.24*	0.24*	0.57***								
VK	Adet	-0.46	0.10	0.20	-0.74**	0.64*	-0.08	0.29							
	Motta	-0.50	0.55*	0.42	-0.32	0.82***	-0.50	0.08							
	combined	0.54***	0.21*	0.16	-0.77***	0.87***	-0.37***	0.28*							
SKHI	Adet	-0.43	0.20	0.08	-0.55*	0.89**	-0.16	0.04	0.69*						
	Motta	-0.20	0.55*	0.55*	-0.31	0.71*	-0.45	-0.03	0.72*						
	combined	0.58***	0.16	0.21*	-0.76***	0.90***	-0.43***	0.21*	0.88***						
SKDM	Adet	0.62*	0.82**	0.15	0.31	-0.25	-0.33	-0.44	0.04	-0.06					
	Motta	-0.04	0.64*	0.32	0.11	0.65*	-0.52*	-0.10	0.59*	0.41					
	combined	0.29*	0.70***	0.24*	-0.06	0.29*	-0.28*	-0.04	0.25*	0.23*					
SKWT	Adet	0.61*	0.81***	0.10	-0.18	-0.16	-0.26	-0.35	0.16	0.14	0.71*				
	Motta	-0.10	0.70*	0.36	0.15	0.65*	-0.50	-0.03	0.63*	0.40	0.92***				
	combined	0.30*	0.80***	0.19	-0.15	0.31*	-0.26*	0.05	0.31*	0.26*	0.93*				
	LFV							0.74***				0.13	0.04	0.11	0.14
	BFLY											-0.43	1	0.91**	-0.26

VK^2, BFLY, FLY^2, FPC^2, from blended samples of the three reps;
FLY^2, BFLY, Buhler extraction method;
LFV and BFLY correlations from mean values (n =15);
GYD-SKDM from raw data (n = 90)

*, **, *** *indicates significant at P<0.05, P<0.01 and P<0.001, respectively*

Mixing time, as a powerful physical dough test, was selected to predict the gluten strength (Bendelow, 1967; Martinant, 1998) and further selection of the best traits describing MDT was made by stepwise regression analysis. The assumption of normality was acceptable. The statistical significance level of 0.05 was used for inclusion of the variable in the prediction. The ANOVA of multiple regression of the linear model and coefficients of its components were significant (Table 29) and the coefficients were within acceptable confidence limits (0.05). Flour protein content, SDSS and SKWT were selected to describe MDT and explained 69 % of the variation using the following prediction equation:

$$MDT = 6.899 - 0.384*FPC + 0.107*SDSS - 0.063*SKWT$$

Flour protein content and SDSS equally contributed to the variation of MDT as their values of partial R^2 and R^2 values was similar during omission of each separately (Table 29). Similarly, their partial correlations with MDT (effects adjusted for the rest of the traits) were equally strong. The contribution of SKWT was lower. The variance inflation ratio and condition number were the minimum which indicate the data has no multicolinearity problem i.e., near linear relationship between independent variables was absent which can cause inaccurate estimates of regression coefficients and degrade predictability. The predictability of the model was assessed using the closeness of R^2 with calculated PRESS R^2. The MDT was predicted using the model and compared with the actual values. The prediction error varied between 0.1 to 13.6 % (in both directions) with only some lines showing sensitivity (Figure 13). Generally, the model under-predicted higher and over-predicted lower values of MDT.

Table 29. Regression coefficients, R^2 and tests of multicolinearity

		R^2 Section				Multicollinearity Section	
Independent Variable	Regression Coefficient	Cumulative Sequential	Partial (Adj.for Rest)	When Omitted	Partial Correlation	Variance Inflation	Condition Number
Intercept	6.899***						
FPC	-0.384***	0.28	0.53	0.34	-0.73	1.14	1.0
SDSS	0.107***	0.65	0.54	0.32	0.74	1.07	1.4
WEIGHT	-0.063**	0.69	0.10	0.65	-0.32	1.07	2.1
R^2 (full model)		0.69					
PRESS R^2		0.66					
Model (ANOVA)	33.4***						

,, the regression coefficient is significant at 0.05 and 0.001*

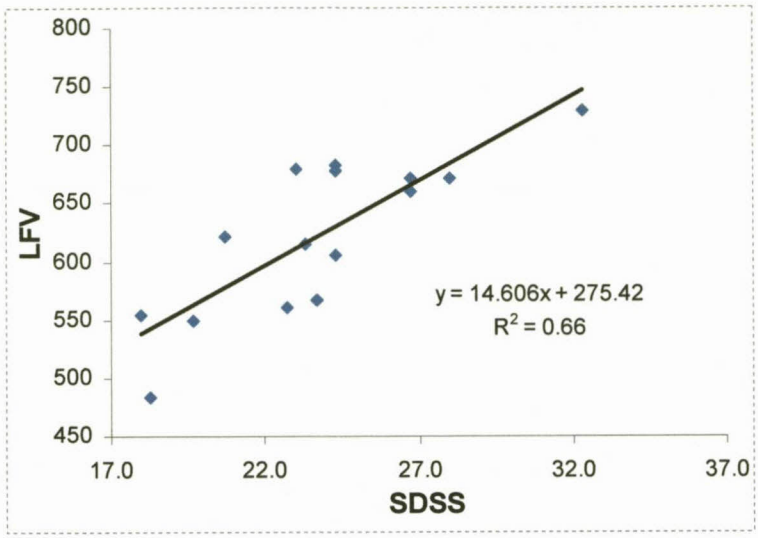


Figure 12. Relationship between SDSS and LFV at higher protein environment

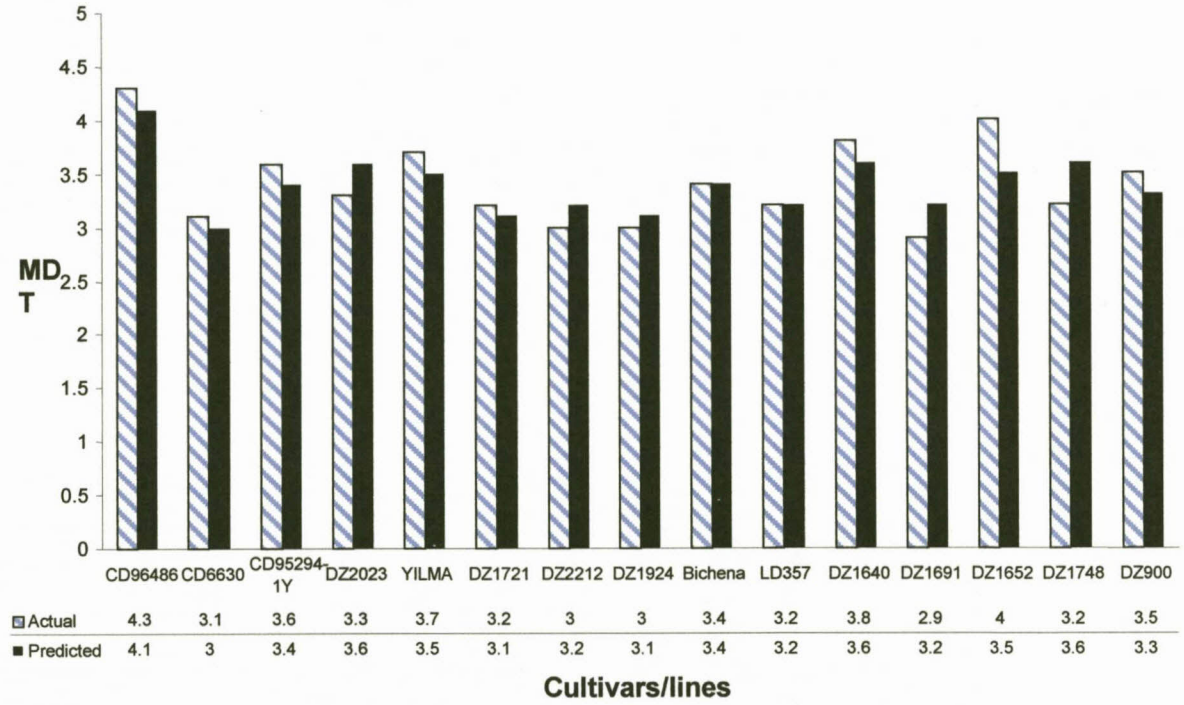


Figure 13. Actual and predicted values of MDT using selected variables in the model

Significant genotypic differences were observed in FPC and VK. The non-significance differences between genotypes for other traits could be due to predominance of environmental and error variation within the sites, or their narrow genetic backgrounds (or both) or the genotypes were not originally selected for differences in quality. Approximately 24 % of the

durum wheat produced worldwide, and up to 70-90 % in some middle-Eastern countries, is used in small bread baking operations (Quaglia, 1988). The bread making quality of durum wheats is very important in Ethiopia as the local produce is consumed in the form of local bread where the fermented dough is baked on traditional hot clay or metal plates. Any variety intended for this use must fit this system of baking in addition to pasta products. Bread volume, color, texture and overall appearance are taken as criteria. Higher loaf volumes (671-730 cm3) were achieved at protein levels of above 10 %, SDSS volume of greater than 23 ml, and mixing time of over 2.7 min. The mixing time increased with decreasing levels of protein as it was shown with negative correlations between the two traits. Therefore, optimum protein levels should be determined for required mixing time to achieve intended end use. Generally, the higher the SDSS volume the higher loaf volume, as they were positively correlated (0.74). Protein content showed weaker but positive association with loaf volume in the analysis using blended samples.

Associations between quality parameters are of great interest in defining optimal values of grain quality for a particular region and to help breeders to produce varieties with good quality (Rharrabti et al., 2003). The difference between the two sites in protein content level was high. Overall site mean of SKHI was much lower at Motta (48.1) than at Adet (67.6). Vitreousness or SKHI had a strong relationship with FPC and selection of varieties maintaining higher percentage of vitreous kernels could be an option to obtain higher FPC. Hardness index was strongly correlated to FPC than VK and only SKHI can be used as a measure of grain hardness. Reduced vitreousness or presence of starchy kernels (yellow berry) reduces semolina (not flour) yield and negatively affects end use quality because starchy parts of the kernels have less protein content than vitreous kernels (Matveef, 1963; Peña et al., 2002) which occurs in a low protein environment. Therefore, a high percentage of vitreousness is required for production of pasta products. Grain hardness is also associated with the milling properties of wheat (Miller et al., 1982; Finney et al., 1987), with water absorption of flour/semolina and with baking quality of the resulting dough. The flour yield was higher at Motta than at Adet because the vitreousness of the grains was very low i.e., more starchy kernels which yielded more flour (Matveef, 1963). Seed diameter, SKWT and TGW had strong positive association with each other at separate and combined environments. SDSS and MDT were positively correlated in both environments but the relationship was stronger at the lower protein environment.

The three selected traits for the model for explaining MDT were weakly correlated to each other indicating their independence and their true separate contribution to explain MDT in this model. There is universal agreement that protein content is the primary factor of quality and gluten strength is the secondary factor in durum wheat (D'Egidio et al., 1990). SDS-sedimentation is an effective rapid indicator of gluten strength (Dexter et al., 1980). The model captured both FPC and SDSS as the major contributors for MDT variation. The third trait, SKWT, had a relatively small effect but contributed more than the rest of the characters. Under medium to very low protein levels and associated effects, the model explained 69 % of the variation of MDT and these parameters could be used for selection. The baking test is considered as the final and most reliable test of bread making. The bread loaf volume of Ethiopian durum wheat genotypes was relatively high at the current protein level indicating that even higher bread volume can be achieved through selection in the future i.e., the protein content must be ≈13 % for leavened bread making (Quaglia, 1988).

Crop management by quality interaction is of critical importance, and agronomic practices suitable for both visual field selection and identification of quality attributes must be employed (Peña et al., 2002). The low protein content at Motta was caused by agronomic factors and needs investigation of optimum agronomic management to increase the protein levels of genotypes.

6.5 Conclusion

It appears that increasing protein content to optimum level could lead to increased gluten strength and quality under both environments. At the low protein environment, vitreousness was significantly reduced, the mixing time increased and the SDSS was reduced which indicated that low FPC affected most quality related traits and optimum protein levels should be determined for intended end products and should be bred accordingly for possible use in an area. The relationship of MDT vs. FPC; FPC vs. VK/SKHI; MDT vs. SKHI; and VK/SKHI vs. FLY were maintained at the low and high protein environments. The absence of G x E interaction for traits was rewarding to make similar selections for both locations. However, sufficient number of sites (years) and genotypes must be sampled and studied for characterization of environments in the region and establish quality criteria for bread and other pasta products. Therefore, the breeding strategy should combine agronomically important traits such as high yield, disease resistance, better adaptability and quality criteria. Releasing a quality variety fulfilling the current demands of consumers and processors will benefit the

predominant small scale wheat growers, encourage domestic production of raw materials for agro industries which can fetch good market prices and enhance self sufficiency using the existing potential. Generally, the region or the country must give emphasis to both agronomic and quality breeding.

6.6 References

AACC (American Association of Cereal Chemists), 2000. Approved methods of the AACC. 10th edition. The association: St. Paul, MN.

Bechere, E., Peña, R.J. and Mitiku, D., 2001. Gluten composition, quality characteristics and agronomic attributes of durum wheat cultivars released in Ethiopia. *African Crop Sci. J.* 10(2):173-182.

Becker, W.A., 1985. Manual of quantitative genetics. Fourth edition. McNaughton and Gunn, Inc. Ann Arbor, Michingan, USA. *p.*48-52.

Bendelow, W.M., 1967. Early generation screening for gluten strength in durum wheat breeding. *Cereal Sci. Today.* 12:366-367.

Boggini, G. and Pogna, N.E., 1989. The bread making quality and storage protein composition of Italian durum wheat. *J. Cereal Sci.* 9:131-138.

D'Egidio, M.G., Mariani, B.M., Nardi, S., Novaro, P. and Cubadda, R., 1990. Chemical and technological variables and their relationships: A prediction equation for pasta cooking quality. *Cereal Chem.* 67:275-281.

D'Agostino, R.B., Belanger, A. and D'Agostino, R.B.Jr. 1990. A suggestion for using powerful and Informative Tests of Normality. *The American Statistician* 44(4):316-321.

Demissie, A, H/mariam, G. and Feyissa, R., 1990. Ethiopian wheat germplasm collection, conservation, characterization/evaluation and utilization. In: Tanner, D.G., van Ginkel, M. and Mwangi, W. *(Eds.).* 1990. Six regional wheat workshop for eastern, Central and Southern Africa. Mexico, D.F.: CIMMYT.

Dessalegn, T. and Wondale, L. 1998. Productivity potential of Durum wheat varieties on well-drained soil. *In:* CIMMYT. 1999. The Tenth Regional Wheat Workshop for Eastern, Central and Southern Africa. Addis Ababa, Ethiopia: CIMMYT. *pp.* 571-574.

Dexter, J.E., Matsuo, R.R., Kosmolak, F.G., Leisle, D. and Marchylo, B.A., 1980. The suitability of SDS-sedimentation test for assessing gluten strength in durum wheat. *Can. J. Plant Sci.* 60:25-29.

Dudley, J.W. and Moll, R.H., 1969. Interpretation and use of estimates of heritability and genetic variances in plant breeding. *Crop Sci.* 9: 257-262.

Finney, K.F, Yamazaki, W.T., Youngs, V.L. and Rubenthaler, G.L., 1987. Quality of hard, soft and durum wheats. *In*: Heyne, E.G. (*Ed.*).Wheat and wheat improvement (2nd edition), ASA Inc., Madison, WI. *pp.*677-748.

Jain, S.K., Qualset, C.O., Bhatt, G.M. and Wu, K.K., 1975. Geographical pattern of diversity in a world collection of durum wheats. *Crop Sci.* 15:700-704.

Liu, C.Y, Shepherd, K.W. and Rathjen, A.J., 1996. Improvement of durum wheat pastamaking and bread making qualities. *Cereal Chem.* 73(2):155-166.

Martinant, J.P., Nicolas, Y., Bouguennec, A., Popineau, Y., Saulnier, L. and Branlard, G., 1998. Relationship between Mixograph parameters and indices of wheat grain quality. *J. Cereal Sci.* 27:179-189.

Matveef, M., 1963. Le mitadinage des blés durs, son évaluation et son influence le rendement et la valuer des semoules. Bull. Anc. Elèves Ecole Franç. *Meunerie* 198:299-306.

Miller, B.S., Afework, S., Pomeranz, Y., Bruinsma, B. and Booth, G.D., 1982. Measuring the hardness of wheat. *Cereal Foods World*, 27:61-64.

Morris, R., Schmidt, J.W., Mattern, P.J. and Johnson, V.A., 1968. Quality tests on six substitution lines involving "Cheyenne" wheat chromosomes. *Crop Sci.* 8:121-123.

NCSS, 2001. Number Cruncher Statistical System, Statistical System for Windows. Jerry L. Hintze. Kaysville, Utah.

Negassa, M., 1986. Estimate of phenotypic diversity and breeding potential of Ethiopian wheats. *Hereditas* 104:41-48.

Payne, T.S, Wanjama, J.K and Girma, B., 2001. Eastern African wheat pool. *In*: Bonjean, A.P. and Angus, W.J. (*Eds.*). The wheat world book: A history of wheat breeding. London: Intercept. *pp.* 902-911.

Peña, R.J., Trethowan, R., Pfeiffer, W.H. and van Ginkel, M., 2002. Quality (end-use) improvement in wheat: compositional, genetic, and environmental factors. *In*: Basra, A.S. and Randhawa, L.S. (*Eds.*). Quality improvement in field crops. Food products press, Binghampton, USA. *pp.* 1-39.

Peña, R.J., Zarco-Hernandez, J., Amaya-Celis, A. and Mujeeb-kazi, A., 1994. Relationship between chromosome 1B-encoded glutenin subunits compositions and bread making quality characteristics of some durum wheat (*Triticum turgidum*) cultivars. *J. Cereal Sci.* 19:243-249.

Porceddu, E., 1976. Variation for agronomical traits in a world collection of durum wheats *Zeitschrift für pflanzenzüchtung* 77:314-329.

Quaglia, G.B., 1988. Other durum wheat products. *In*: Fabriani, G and Lintas, C. (*Eds.*). Durum wheat: Chemistry and technology. Am. Assoc. Cereal Chem., St. Paul. MN. *pp.* 263-283.

Rharrabti, Y. Villegas, D. Royo, C. Martos-Nu´ ez, V. and Garcý´a del Moral, L.F., 2003. Durum wheat quality in Mediterranean environments II. Influence of climatic variables and relationships between quality parameters. *Field Crops Res.* 80: 133–140.

SAS, 1994. SAS/STAT ™. Guide for personal computers. Version 6 Edition. SAS institute Inc., Cary, NC.

Schmidt, J.W., Morris, R., Johnson, V.A. and Mattern, P.J., 1966. Comparison of the chromosome substitution and monosomic methods for wheat quality studies. *Crop Sci.* 6:370-372.

Simmonds, D.H., 1989. Inherent quality factors in wheat. In: Wheat and wheat quality in Australia. ©CSIRO. William Books Qld., 2:37-38.

Singh, M., Ceccarelli, S. and Hamblin, J., 1993. Estimation of heritability from variety trials data. *Theor. Appl. Genet.* 86:437-441.

Tessema, T., 1987. Durum wheat breeding in Ethiopia. *In*: Van Ginkel, M. and Tanner, D.G. *(Eds.)*. Fifth regional wheat workshop for Eastern, Central and Southern Africa and the Indian Ocean. Mexico, D.F.: CIMMYT. *pp.* 18-22.

Vavilov, N.I., 1951. The origin, variation, immunity and breeding of cultivated plants. *Chronica Botanica* 13:1-366.

Vavilov, N.I, 1931. The wheat of Abyssinia and their place in the general system of wheats, Bulletin of Applied Botany, Genetics, and Plant Breeding, Supplement 51, *p.*233.

Chapter 7 B-LMW glutenins and γ-gliadin compositions of Ethiopian durum wheat genotypes and their association with some quality traits

7.1 Abstract

Thirty advanced lines and cultivars were investigated for their specific B-LMW glutenins (LMW-1 and LMW-2) and γ-gliadin (γ-42 and γ-45) composition by SDS-PAGE analysis and their relationship to SDS-sedimentation volume (SDSS), mixograph development time (MDT) and bread loaf volume (LFV). The proportion of lines and cultivars with LMW-2/ γ-45 type subunits was much higher than LMW-1/ γ-42 types. Lines possessing LMW-2 / γ-45 type compositions achieved higher SDSS, MDT and LFV values. The highest bread volume (730 ml) was recorded by line CD96486 having subunits N, 7+8 and LMW-2 / γ-45. It had better expression of SDSS and MDT than the rest of the genotypes. The high proportion of LMW-2 / γ-45 type compositions in the advanced lines, which is genetically related to good grain quality, is an advantage to select suitable genotypes for intended products. Selection for higher values of SDSS and MDT would be necessary to achieve better bread volume. Further studies should also include selecting genotypes suitable for pasta products to meet the domestic demands. Screening of genotypes using genetic markers of wheat storage proteins related to quality will help to devise an effective breeding program for the region and to build a database for variety identification and diversification.

Key words: B-LMW glutenins,Durum wheat, γ-gliadins, quality traits, SDS-PAGE

7.2 Introduction

Durum wheat (*Triticum turgidum* L. (Thell.) *ssp. Durum* (Desf.) Husn.) is a widely grown ancient crop in Ethiopia. It is commonly used for traditional food recipes in addition to pasta products. The breeding effort in the country placed greater emphasis on high yield and disease resistance. However, the feedback from producers, processors and consumers indicated the need to strengthen breeding for improved nutritional quality (Bechere et al., 2001; Tadesse, unpublished survey information, 2001). The quality of durum wheat refers to its suitability for the intended end use (Troccoli et al., 2000) and criteria depend on the various aspects of growth and utilization. Pasta product quality depends on gluten quality and protein content

(Feillet et al., 1989; Troccoli et al., 2000) with the effects of genotype, environment and GxE interaction on these parameters. Durum wheat semolina for bread making needs to have gluten that is less elastic and more extensible (Boggini and Pogna, 1989; Liu et al., 1996) and some varieties have better bread making quality than others. Variations in gluten strength is usually attributed to differing aggregative behavior among glutenins i.e., high molecular weight (HMW) or A subunits coded at Glu-1 locus and low molecular weight (LMW) glutenins or B and C subunits coded at Glu-3 (MacRitchie, 1992). Genetic markers of quality are used to evaluate and select lines in early generations. Consistent relationships were found between the presence of specific LMW proteins and gluten quality (Payne et al., 1984; Pogna et al., 1990). Two subunits were designated as LMW-1 and LMW-2 related to poor and good gluten strength, respectively. The presence of γ-42 and γ-45 gliadins bands at the Gli-B1 loci were used as genetic markers of poor and good quality, respectively (Kosmolak et al, 1980). However, these gliadin bands were not directly responsible but markers tightly linked with LMW glutenins, LMW-1 and LMW-2, which had direct effects on quality (Payne et al., 1984; Ruiz and Carrillo 1993). The HMW-GS of durum wheat has little effect on gluten quality (Pogna et al., 1990) although some significant variation has been detected at Glu-B1 encoded proteins.

Northwestern Ethiopia has the potential for extensive and intensive durum wheat production and it is among the leading durum wheat producing regions of the country (Dessalegn and Wondale, 1998). The objective of this study was to investigate the presence of genetic markers of HMW-GS, LMW-GS and gliadin components in a group of Ethiopian advanced lines and cultivars tested in the region and its relationship with grain quality..

7.3 Material and methods

Thirty genotypes were analyzed using the one-step one-dimensional sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) separation as described by (Singh et al., 1991) on 10 % polyacrylamide gels. Six randomly picked seeds from a well mixed grain sample were analyzed for each cultivar/line and compared with standards of known bands from Canada and Spain. The standards were Mexicali (LMW-2, γ-45), Langdon (LMW-1, γ-42), Alaga (γ-44) and Marquis (R50) and Chinese Spring (7+8; 2+12). The presence of LMW-1/ γ-42 and LMW-2/γ-45 variants was checked for those genotypes showing uniformity in HMW glutenin subunits. The nomenclature of Payne et al. (1983) was used to identify the high molecular weight subunits. LMW and gliadin variants were identified as described by Carrillo et al. (1991) and Bushuk and Sapirstein (1991), respectively. The subset of 15 genotypes (3

cultivars and 12 lines) were grown in a RCB design in three replications at two locations (Adet and Motta) in northwestern Ethiopia and some physicochemical and rheological traits were investigated (Table 30).

Table 30. Methods, units and abbreviations of measurements of quality traits

Traits	Abbreviations	Unit	Methods of measurements
Flour protein content	FPC	%	AACC 39-11
SDS-sedimentation test	SDSS	ml	AACC 56-70
SKCS- hardness index	SKHI		AACC 53-31
Mixograph development time	MDT	min	AACC 54-40A
Loaf volume	LFV	cm^3	AACC 10-09

AACC, American Association of Cereal Chemists (2000); SKCS, Single Kernel Characteristics System

7.4 Results and discussion

The uniformity of each genotype was checked at the Glu-1 locus and five advanced lines which showed polymorphism within samples were not considered for further analysis of LMW and gliadins (Table 31). The heterogeneity was at Glu-B1 and each cultivar had 2-5 different subunit mixtures. Generally, two and seven alleles were identified at Glu-A1 and Glu-B1 loci, respectively, in all studied genotypes (Table 32). At Glu-A1, 98 % of the genotypes had the null subunit as most varieties in durum wheat posses the same allele (Branlard et al., 1989). Subunit 7+8 (48%) was dominant at Glu-B1 followed by subunit 20 (32 %) and 6+8 (16 %). Subunits 7+9 and 20 were found in single cultivar each. The rest of the subunits (7; 14+15 and 13+16) were observed in advanced lines having mixtures,

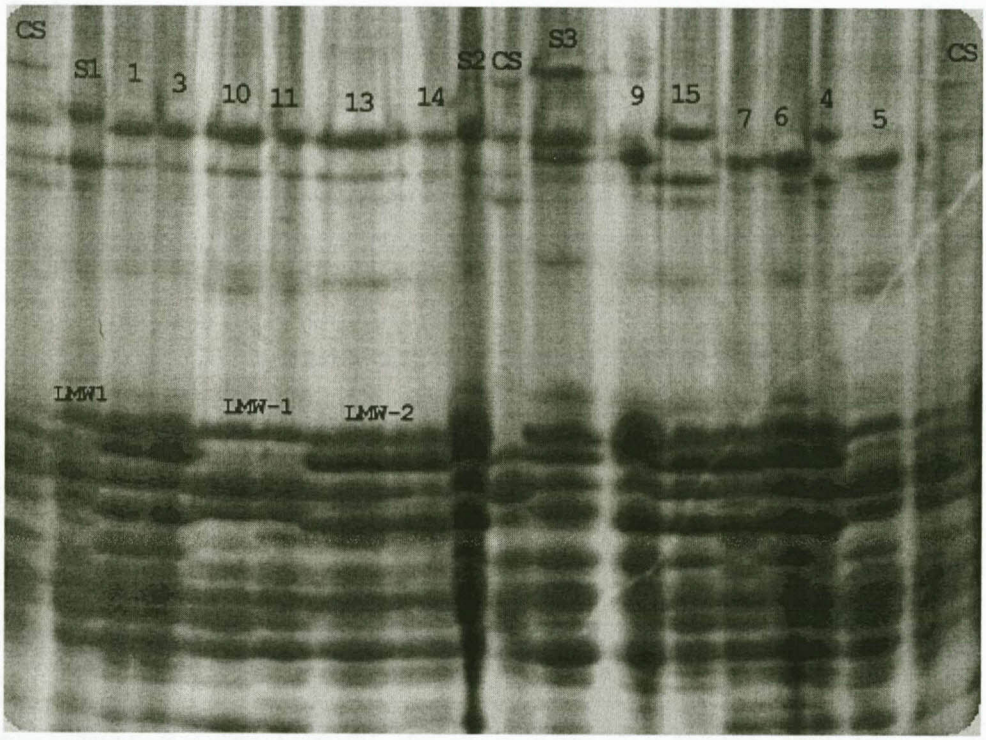
Table 31. Patterns and allelic compositions of HMW, B-LMW and γ-Gliadins of Ethiopian genotypes and standards

		HMW(Glu-1) subunits		γ-Gliadins	LMW models
No	Name	A1	B1	(Gli-A1)	(Glu-B3)
1	CD96486	N	7+8	45	LMW-2
2	CD6630	N	7+8	45	LMW-2
3	CD95294-1Y	N	7+8	45	LMW-2
4	DZ2023	N	6+8	45	LMW-2
5	Yilma	N	20	42	LMW-1
6	DZ1721	N	20	45	LMW-2
7	DZ2212	N	20	45	LMW-2
38	DZ1924[§]	N	6+8;14+15		
9	Bichena	N	20	45	LMW-2
10	LD357	N	7+8	42	LMW-1
11	DZ1640	N	7+8	42	LMW-1
12	DZ1691[§]	N	17+18; 6+8;7+9		
13	DZ1652	N	7+8	45	LMW-2
14	DZ1748	N	7+8	45	LMW-2
15	DZ900	N	6+8	45	LMW-2
16	CD94022-4Y	N	20	42	LMW-1
17	CD94545	2*	20	45	LMW-2
18	CD95526	N	7+9	45	LMW-2
19	CD95437	N	7+8	45	LMW-2
20	DZ1928-1	N	7+8	45	LMW-2
21	CD95759-11M	N	7+8	45	LMW-2
22	DZ2234	N	6+8	45	LMW-2
23	CD95731	N	6+8	45	LMW-2
24	CD96643-5Y	N	7+8	45	LMW-2
25	CD95294-2Y	N	20	45	LMW-2
26	CD95324	N	20	45	LMW-2
27	CD96643-3Y	N	7+8	45	LMW-2
28	CD9643-6Y[§]	N	7;6+8		
29	CD2101[§]	N	7+8; 13+16		
30	DZ1838[§]	N	6+8; 14+15; 7+8; 7+9		

[§], *heterogeneous at Glu-B1 locus*

Table 32. Frequency of glutenin and gliadin subunits composition

Locus	Allele	Subunit	No. of genotypes	Frequency (%)
Glu-A1	c	null	24	96
	b	2*	1	4
Glu-B1	b	7+8	12	48
	e	20	8	32
	d	6+8	4	16
	c	7+9	1	4
	a	7		
	h	14+15		
	f	13+16		
Gli-B1/Glu-A3		LMW-2/ γ-45	22	88
		LMW-1/ γ-42	3	12



Standards S1,Langdon; S2, Mexicali; S3,Alaga; CS, Chinese spring;
Numbers 1-15 are test entries
Figure 14. SDS-PAGE subunit patterns of HMW and B-LMW glutenins

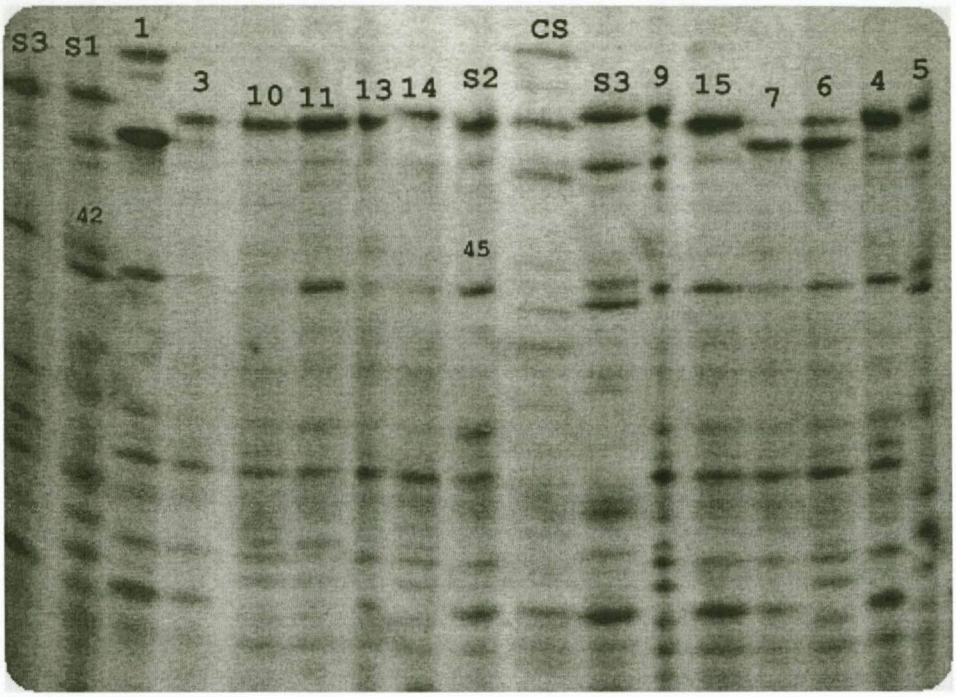


Figure 15. SDS-PAGE patterns of gliadin in some of the genotypes

Twenty five out of 30 genotypes were analyzed for B-LMW and γ-gliadin compositions (Table 31). Twenty two genotypes (88 %) possessed LMW-2/γ-45 type subunits and three (12 %) with LMW-1/γ-42 subunits. The two LMW-models (LMW-1 and LMW-2) and γ-gliadin variants are shown in Figure 14 and 15.

A subset of 15 genotypes i.e., 11 with LMW-2 (γ-45), two with LMW-1 (γ-42) and two heterogeneous genotypes (mixed HMW patterns) were grown at two locations for their gluten strength and bread making quality. The result of the measured traits is presented in Table 33.

Table 33. Mean of some quality traits of studied lines and cultivars across two environments

No	Name	HMW (Glu-1) subunits A1	HMW (Glu-1) subunits B1	LMW Models	γ-Gliadins	FPC	MDT	SDSS	SKHI	LFV‡
1	CD96486	N	7+8	LMW-2	45	9.1	4.3	26.8	55.3	730
2	CD6630	N	7+8	LMW-2	45	10.3	3.1	21.8	63.0	678
3	CD95294-1Y	N	7+8	LMW-2	45	9.3	3.6	21.7	60.1	615
4	DZ2023	N	6+8	LMW-2	45	8.6	3.3	22.0	53.3	683
5	Yilma	N	20	LMW-2	45	9.9	3.7	25.2	62.1	561
6	DZ1721	N	20	LMW-2	45	9.1	3.2	18.7	57.6	672
7	DZ2212	N	20	LMW-2	45	9.8	3.0	22.3	60.2	606
8	DZ1924§	N	6+8; 14+15			9.7	3.0	20.8	61.7	661
9	Bichena	N	20	LMW-2	45	8.9	3.4	22.3	55.0	679
10	LD357	N	7+8	LMW-1	42	8.6	3.2	16.5	54.5	555
11	DZ1640	N	7+8	LMW-1	42	8.4	3.8	20.3	48.9	549
12	DZ1691§	N	17+18; 6+8;7+9			9.6	2.9	21.0	59.0	567
13	DZ1652	N	7+8	LMW-2	45	9.3	4.0	22.2	57.2	621
14	DZ1748	N	7+8	LMW-2	45	9.5	3.2	25.8	61.3	671
15	DZ900	N	6+8	LMW-2	45	9.0	3.5	19.7	58.7	483
CV (%)						10.4	34.9	35.0	12.3	
LSD(0.05)						0.9	1.1	7.4	6.9	

‡, *measured from blended samples of the highest protein site, Adet*

Table 34. Mean values of quality traits for glutenin and gliadin patterns

Patterns	FPC	MDT	SDSS	SKHI	LFV
Null; 7+8	9.2	3.6	22.2	57.2	631.3
Null; 20	9.4	3.3	22.1	58.7	629.5
Null; 6+8	8.8	3.4	20.9	56.0	583.0
LMW-2 /γ-45	9.4	3.4	22.3	58.8	632.8
LMW-1/ γ-42	8.5	3.5	18.4	51.7	552.0

The studied Ethiopian cultivars and advanced lines had a high frequency of LMW-2/γ-45 related glutenin/gliadin subunits as in most durum genotypes in many breeding programs (Lin and Shepherd, 1996). There was no deliberate selection for accumulation of such specific bands (Bechere et al., 2001) and the frequencies were random during selection for agronomically superior genotypes, which was expected to have no effect on the observed frequencies. The frequency of LMW-1/γ-42 related subunits was very low (12 %). Cultivars possessing LMW-2/γ-45 subunits had a higher mean FPC, SDSS, SKHI and LFV than those with LMW-1/γ-42 subunits and similar expression of MDT. However, the values of LMW-2/γ-45 ranged from intermediate to high due to lines having γ-45 exhibiting different alleles (a,c,d,e,f,and g) at Glu-B3 loci (Neito-Taladarez, 1997). Many studies confirmed that the presence of LMW-2/γ-45 was related to better gluten strength and suitability to various end

uses (Payne et al., 1984; Carrillo et al., 1991). Generally, the mean protein content of the trials was low (8.4-10.3 %) but the expected genetic expression of the two LMW-models was maintained.

In durum wheats, HMW glutenin subunits were not found to have a strong effect on pasta quality (Autran and Galterio, 1989). However, it was reported that certain subunits such as 7+8 at Glu-B1 is associated with better gluten strength than 6+8 or 20 (Pogna et al., 1990). In this study, the mean MDT, SDSS and LFV were slightly higher for genotypes with 7+8 than those with 6+8 and 20 subunits (Table 34). Line CD96486 possessing N; 7+8 and LMW-2 / γ-45 combinations which could be due to additive effects of HMW and LMW glutenins (Fares et al, 1997) had the highest LFV (730 ml). The same cultivar had the highest MDT and SDSS values. The two heterogeneous lines at Glu-B1 locus did not show any advantage over pure lines for most quality traits.

7.5 Conclusion

The polymorphism was low at Glu-A1 locus and higher at Glu-B1 in these set of genotypes. Most of the cultivars had LMW-2/γ-45 which would facilitate selection for better performance of required gluten strength. Selection for higher values of SDSS and MDT would be necessary to achieve better bread volume and further studies should include selecting genotypes suitable for pasta products to meet the domestic demands. The proportion of heterogeneous advanced lines (17 %) was large and attention should be given to fix genotypes for promotion in advanced trials. Screening of genotypes with better quality using markers of wheat storage proteins related to quality will help to devise an effective breeding program for the region and to build a database for variety identification and diversification . In addition, other factors such as genotypic and environmental conditions affecting endues quality should be also investigated.

7.6 References

AACC (American Association of Cereal Chemists), 2000. Approved methods of the AACC. 10th edition. The association: St. Paul, MN.

Autran, J.C. and Galterio, G., 1989. Associations between electrophoretic composition of proteins, quality characteristics and agronomic attributes of durum wheat. II. Protein quality association. *J. Cereal Sci.* 9:195-215.

Bechere, E., Peña, R.J. and Mitiku, D., 2001. Gluten composition, quality characteristics and agronomic attributes of durum wheat cultivars released in Ethiopia. *African Crop Sci. J.* 10(2):173-182.

Boggini, G. and Pogna, N.E., 1989. The bread making quality and storage protein composition of Italian durum wheat. *J. Cereal Sci.* 9:131-138.

Branlard, G., Autran, J.C. and Monneveux, P., 1989. High molecular weight glutenin subunits in durum wheat (*Triticum durum*). *Theor. Appl. Genet.* 78:353-358.

Bushuk, W., and Sapirstein, H.D., 1991. Modified nomenclature for gliadins. *In*: Bushuk, W. and Tkachuk, R. (*Eds*). Gluten Proteins. Am. Assoc. Cereal Chem.: St. Paul, MN. *pp.* 454-458.

Carrillo, J.M., Vazquez, J.F., Ruiz, M. and Albuquerque, M.M., 1991. Relationship between gluten strength and protein components in Spanish durum wheat landraces. *In*: Bushuk, W. and Tkachuk, R. (Eds.). Gluten proteins. 1990. AACC, Inc., St.Paul, Minnesota, USA.*pp.*268-276.

Dessalegn, T. and Wondale, L. 1998. Productivity potential of Durum wheat varieties on well-drained soil. *In:* CIMMYT. 1999. The Tenth Regional Wheat Workshop for Eastern, Central and Southern Africa. Addis Ababa, Ethiopia: CIMMYT. *pp.* 571-574.

Fares, C., Novembre, G., Di Fonzo, N., Galterio, G. and Pogna, N.E., 1997. Relationship between storage protein composition and gluten quality in breeding lines of durum wheat (*Triticum turgidum ssp. Durum). Agricultura Mediterranea 197:363-368.*

Feillet, P., Ait-Mouh, O., Kobrehel, K. and Autran, J.C., 1989. The role of low molecular weight glutenin proteins in the determination of cooking quality of pasta products: an overview. *Cereal Chem.* 66:26-30.

Kosmolak, F.G., Dexter, J.E., Matsuo, R.R., Leslie, D., and Marchylo, B.A., 1980. A relationship between durum wheat quality and gliadin electrophoregrams. *Can. J. Plant Sci.* 60:427-432.

Lin, C.Y. and Shepherd, K.W., 1996. Variation of B subunits of glutenin in Durum, wild and less-widely cultivated tetraploid wheats. *Plant Breed.* 115:172-178.

Liu, C.Y, Shepherd, K.W. and Rather, A.J., 1996. Improvement of durum wheat pasta making and bread making qualities. *Cereal Chem.* 73(2):155-166.

MacRitchie, F., 1992. Physicochemical properties of wheat proteins in relation to functionality. *Adv. Food Nutr. Res.* 36:1-87.

Neito-Taladriz, M.T., Ruiz, M., Martinez, M.C.M, Vazquiz, J.F. and Carrillo, J.M., 1997. variation and classification of B low molecular weight glutenin subunit alleles in durum wheat. *Theor. Appl. Genet.* 97:1155-1160.

Payne P.I. and Lawrence G.J., 1983. Catalogue of alleles for the complex gene loci, Glu-A1, Glu-B1 and Glu-D1 which code for high molecular weight sub units of glutenins in hexaploid wheat. *Cereal Res. Commun.* 11:29-35.

Payne, P.I., Jackson, E.A. and Holt, L.M., 1984. The association between γ-gliadin 45 and gluten strength in durum wheat varieties: A direct causal effect or the result of genetic linkage? *J. Cereal Sci.* 2:73-81.

Pogna, N.E., Autran, J.C., Mellini, F., Lafiandra, D. and Feillet, P., 1990. Chromosome 1B-encoded gliadins and glutenin subunits in durum wheat: genetics and relationship to gluten strength. *J. Cereal Sci.* 11:15-34.

Ruiz, M. and Carrillo, J.M., 1993. Linkage relationships between prolamin genes on chromosome 1A and 1B of durum wheat. *Theor. Appl. Genet.* 87:353-360.

Singh N.K., Shepherd and Cornish G.B., 1991. A simplified SDS-PAGE procedure for separating LMW subunits of glutenins. *J. Cereal Sci.* 14:203-208.

Troccoli,A., Borrelli, G.M., De.Vita, P., Fares, C. And Di Fonzo, N., 2000. Durum wheat quality: a multidisciplinary concept. *J.Cereal Sci.* 32:99-113.

Chapter 8 Summary and recommendations

Summary

- Ethiopian cultivars and advanced lines of bread and durum wheat, originally selected for agronomic superiority, were studied for their grain quality parameters under different environments. Direct and indirect measures of quality were used to establish prediction equations of quality to recommend the minimum quality tests to be used in the breeding program of northwestern Ethiopia.

- The HMW-GS of bread and durum wheat, the B-LMW and γ-gliadin of durum wheat protein subunit compositions were identified and their associations with quality traits were studied. The combining ability, relative importance of additive and non-additive gene effects, genetic associations and inheritance of grain and quality traits were also investigated.

Bread wheat

- Genetic variability was detected among genotypes studied in most important quality traits. The loaf volume, which is the final product, ranged between 882.2 to 1010.8 cm^3 with protein levels of 10.7-14.3%. The protein content levels of genotypes at the two Ethiopian sites were relatively lower. The mean mixing time increased (up to 5.5 min) with decreasing protein content of genotypes i.e. genotypes grown at the lowest protein site had the longest mixing time.

- Among 15 independent grain yield and quality traits studied, flour protein content, SDS-sedimentation test, P/L ratio, gluten content and thousand grain weights explained 73 % of the variation in loaf volume under the higher protein (10-14 %) environment. The variation of mixograph development time due to protein content alone was 37 % at the lower protein environment.

- Highly repeatable traits were P/L ratio, break flour yield, SDS-sedimentation volume, hardness index, mixing time and loaf volume ranging from 0.63 to 0.91.

- Loaf volume had a significant positive correlation with mixograph development time, SDS-sedimentation volume and alveograph gluten strength.

- In a combining ability analysis, most studied traits showed significant additive gene effects. The ratio of GCA: SCA was 0.7 to 37. Heritability of mixograph development time (0.81), SDS-sedimentation volume (0.73), hardness index (0.68), grain weight (0.57) and flour yield (0.46) was higher than the rest of the traits. In studied sets of parents, utilizing additive genetic variance would be effective to improve mixing time, SDS-sedimentation volume, flour yield, vitreous kernels and thousand grain weights.

- Eleven banding patterns of HMW glutenin subunits i.e., three at Glu-A1, five at Glu-B1 and two at Glu-D1 were identified in Ethiopian bread wheat genotypes. The frequency of subunits 5+10, the high quality scoring alleles, was 78 %. Banding patterns showed significant differences in SDS-sedimentation volume, alveograph gluten strength, and loaf volume. Pattern 1, 7+8, 5+10 gave the best results in the studied genotypes.

Durum wheat

- The loaf volume ranged from 483-730 cm^3 at protein levels of 10.1-12.5 %. Higher loaf volumes could be achieved by selecting for higher SDS-sedimentation volume and protein genotypes. Flour protein content, SDS-sedimentation volume and seed weight explained 69 % of the variation of mixograph development time.

- The frequency of the B-LMW glutenin and γ-gliadin (*LMW-2/ γ-45*) subunit combination of proteins with better gluten strength and loaf volume was high in the studied genotypes. The highest bread volume (730 cm^3) was recorded by line CD96486 possessing subunit combination of N/7+8 at *Glu-A1/B1* loci and *LMW-2/ γ-45* at *Glu-A3* and *Gli-B1* loci, respectively.

Recommendations

Breeding for quality

- Genetic markers of protein are important in pre-determining lines of good or poor grain quality at early and/or late generations and help to accelerate breeding procedures. Introduction and use of protein markers i.e., HMW, LMW and gliadins, which could be separated by SDS-PAGE analysis, could be an advantage to select genotypes possessing good alleles indicating better gluten strength. Further studies of protein composition under different environmental (climatic, soil, altitude, etc.) conditions of the region is required to identify which alleles of glutenin/gliadins, more than others, are genetically associated with grain quality.

- Direct and indirect physical quality tests are vital at early and/or advanced stages of selection depending on the availability of seed required for testing. Based on the genetic control of the traits, observed correlations, heritabilities and predictions, measurements of gluten strength (SDS-sedimentation test, mixing time), protein content and grain hardness (hardness index), among the rest, could be introduced as minimum selection criteria, where resources are limited, in the northwestern Ethiopian wheat-breeding program.

- Protein content can be largely influenced by environment. Therefore, the crop management necessary to maintain protein levels required for specific products should be further studied based on the domestic requirements. Concurrently, varietal breeding for quality should be targeted for the end use product. The wheat growing environments should be characterized according to the grain type and protein levels achievable in each environment. More years and location should be investigated to make efficient predictions of quality, which accommodate location and seasonal effects.

- Ethiopia and/or the region lack complete standards of quality parameters (flour, dough, end-use products, etc), which could be used as a target for all entrepreneurs, involved in wheat industry. Therefore, priority should be given to set the standards of quality

based on local consumer demands, milling and baking requirements, producers and international standard requirements.

- Building a wheat quality laboratory is a prerequisite for any wheat-breeding program in order to understand the enduses of a variety. It will be necessary to measure important direct and/or indirect traits of quality which fulfil the requirments of producers, millers, bakers and end-users.

Hoofstuk 8 Opomming en aanbevelings

Opsomming

- Ethiopiese cultivars en gevorderde teellyne van brood en durum korings wat oorspronklik geselekteer is vir agronomiese meerderwaardigheid, is vir graan- kwaliteit in verskillende omgewings bestudeer. Direkte en indirekte metings van kwaliteit is gebruik om voorspellende vergelykings van kwaliteit op te stel, om 'n aanbeveling te maak betreffende die minimum kwaliteitstoetse vir gebruik in teelprogramme in die noordweste van Ethiopië.

- Die HMG van brood en durum koring, die B-LMG en Y-gliadiene van durum koring proteïen subeenhede samestelling is geïdentifiseer en hul assosiasies met kwaliteits-eienskappe is bestudeer. Die kombineervermoë, relatiewe belangrikheid van additiewe en nie-additiewe gene effekte, genetiese assosiasies en oorerflikhede van graan en kwaliteitseienskappe is bestudeer.

Broodkoring

- Genetiese variabiliteit is gevind tussen genotipes vir meeste van die belangrikste kwaliteitseienskappe. Die broodvolume, wat die finale produk is, varieer tussen 882.2 tot 1010.8 cm^3 met proteïenvlakke van 10.7 – 14.3%. Die proteïenvlakke van die genotipes by die twee Ethiopiese lokaliteite was relatief laer. Die gemiddelde mengtyd het toegeneem (tot 5.5 min) met 'n afname in proteïeninhoud van die genotipes bv. genotipes verbou in die lokaliteit met die laagste proteïenvlak, het die langste mengtye.

- Van die 15 onafhanklike graan en kwaliteitseienskappe wat bestudeer is, het proteïeninhoud, SDS-sedimentasie toets, P/L verhouding, gluteninhoud en duisend-korrelmassa 73% van die variasie vir broodvolume in die hoë proteïen omgewing verklaar. Die variasie in miksogram ontwikkelingstyd a.g.v. proteïeninhoud alleen, was 37% in die lae proteïen omgewing.

- Eienskappe met hoë herhaalbaarhede was P/L verhouding, breekmeelopbrengs, SDS-sedimentasie volume, hardheidsindeks, mengtyd en broodvolume. Die herhaalbaarheid varieer tussen 0.63 en 0.91.

- Broodvolume het 'n betekenisvolle korrelasie met miksogram ontwikkelingstyd, SDS-sedimentasie volume en alveo-gluten sterkte.

- In 'n kombineervermoë ontleding, toon meeste van die bestudeerde eienskappe betekenisvolle additiewe gene effekte. Die verhouding van GCA:SCA het gevarieer tussen 0.7 en 0.37. Oorerflikhede vir mixogram ontwikkelingsytd (0.81), SDS-sedimentasie volume (0,73), hardheidsindeks (0.68), graangewig (0.57) en meelblomopbrengs (0.46) was hoër as die res van die eienskappe. In die bestudeerde stel ouers, is die benutting van die additiewe genetiese variansie effektief om eienskappe soos mengtyd, SDS-sedimentasie volume, meelblomopbrengs, horingagtige korrels en duisendkorrelmassa te verbeter.

- Elf bandpatrone van HMG glutenien subeenhede bv. drie by Glu-A1, vyf by Glu-B1 en twee by Glu-D1 is geïdentifiseer in Ethiopiese broodkoring genotipes. Die frekwensie van subeenhede 5 + 10, die hoë kwaliteitstelling allele, was 78%. Bandpatrone veroorsaak betekenisvolle verskille in SDS-sedimentasie volume, alveograaf glutensterkte en broodvolume. Bandpatrone 1, 7+8, 5+10 het die beste resultate gelewer in die bestudeerde genotipes.

Durum koring

- Die broodvolume varieer van 483-730 cm^3 by proteïenvlakke van 10.1-12.5%. Hoër broodvolumes kan verkry word deur seleksie vir hoër SDS-sedimentasie volume en proteïen genotipes. Meelblom proteïeninhoud, SDS-sedimentasie volume en saadgewig het 69% van die variasie in miksogram ontwikkelingstyd verklaar.

- Die frekwensie van die B-LMG gluteniene en Y-gliadien (LML-2/Y45) subeenhede kombinasie van proteïene met beter gluten sterkte en broodvolume was hoog in die bestudeerde genotipes. Die hoogste broodvolume (730 cm^3) is verkry vanaf lyn CD

96486 wat oor die subeenhede kombinasies van N/7+8 by Glu-A1/B1 loci en LMG-2/Y-45 by Glu-A3 en Gli-B1 loci, respektiewelik.

Aanbevelings

Veredeling van kwaliteit

- Genetiese merkers van proteïen is belangrik vir die voorafbepaling van lyne wat oor goeie of swak kwaliteitskenmerke beskik in vroeëre en latere generasies en help om teelprosedures te versnel. Introduksie en die gebruik van proteïenmerkers bv., HMG, LMG en gliadiene, wat geskei kan word deur SDS-PAGE ontledings, hou voordele in om genotipes te selekteer met voortreflike allele met 'n beter glutensterkte. Verdere studies betreffende proteïen samestelling in verskillende omgewingstoestande (klimaat, grond, hoogte, ens.) van die gebied word benodig om te identifiseer watter allele van gluteniene/gliadiene geneties geassosieer is met graankwaliteit.

- Direkte en indirekte fisiese kwaliteitstoetse is noodsaaklik by vroeëre en gevorderde stadia van seleksie, afhangende van die hoeveelheid saad wat benodig word vir toetsing. Eienskappe soos waargenome korrelasies, oorerflikhede en voorspellings, meting van glutensterkte (SDS-sedimentasie-toets, mengtyd), proteïeninhoud en graanhardheid (hardheidsindeks), kan aangewend word as minimum seleksiekriteria, waar hulpbronne beperk is, in die noordwestelike Ethiopiese koringteelprogram.

- Proteïeninhoud word grootliks deur die omgewing beïnvloed. Gevolglik behoort gewasverbouingsaspekte om die nodige proteïenvlakke vir spesifieke produkte te verkry, bestudeer te word. Huidiglik behoort kwaliteitsverbetering gerig te wees op die eindverbruik van die produk. Die koring produserende gebiede behoort gekarakteriseer te word in ooreenstemming met die graantipe en die proteïenvlakke wat haalbaar is in elke omgewing. Meer jare en lokaliteite behoort bestudeer te word, om effektiewe voorspellings van kwaliteit te maak, wat die lokaliteit en seisoenale effete akkommodeer.

- Ethiopië beskik nie oor die nodige standaarde vir kwaliteitsontledings (meelblom, deeg en eindgebruik produkte), wat gebruik kan word as norme vir alle belanghebbendes in

die koringindustrie. Gevolglik sal prioriteit verleen en standaarde gestel moet word vir kwaliteit, gebaseer op die plaaslike behoefte, maal- en bakvereistes, produsente en internasionale behoeftes.

- Die oprigting van 'n kwaliteitslaboratorium is 'n voorvereiste vir enige koringteelprogram om die kennis te verhoog van enige variëteit en die potensiaal vir 'n spesifieke eindgebruik. Dit is gevolglik noodsaaklik om die minimum direkte en indirekte eienskappe te meet wat benodig word, om variëteite t.o.v. kwaliteit te toets, ten einde behulpsaam te wees om in die behoeftes te voorsien van produsente, bemarkers, meulenaars, bakkers en eindgebruikers.