THE RELATIONSHIP OF SODIUM DODECYL SEDIMENTATION TEST VALUES TO BREADMAKING QUALITY OF EARLY GENERATION DRYLAND WHEAT LINES

RACHEL MERCIA OELOFSE

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Faculty of Natural and Agricultural Sciences
Department of Plant Science (Plant Breeding)
University of the Free State
BLOEMFONTEIN

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SUPERVISOR: **Prof. M.T. LABUSCHAGNE**

CO-SUPERVISOR: **Prof. C.S. VAN DEVENTER**

This work is dedicated to my sister, Lienke Oelofse,
my mother, Marina Oelofse,
and my grandmother, Rae van Heerden.

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CHAPTER 1

INTRODUCTION

Wheat quality requirements are diverse and depend on the type of wheat, expected end product and production environment (Graybosch *et al*., 1996). The South African market consists of hard red wheat utilized for human consumption, mainly as bread. South Africa is a country with diverse production regions considering the extreme variation in the climatic conditions (Aucamp, 2003). Production environments can roughly be divided into three categories:

- Western Cape area, where spring types are produced under rain fed conditions
- Free State area, where winter and intermediate wheat are cultivated under rain fed conditions
- Northern areas, where spring types are cultivated under fully irrigated conditions

In this study, the focus was on wheat quality of the Free State province, where winter and facultative types are planted during the autumn and winter months (April to July) on conserved soil moisture. This region's production contributes approximately 50% of the total wheat production of the country (Aucamp, 2003).

The vegetative growth stage of the plants is entirely dependent on conserved soil moisture. Correct production practices to conserve the moisture is therefore of utmost importance. The summer rainfall period extends from October to March. Plants are mostly already in the reproductive growth stage at the beginning of this period. Mostly no supplementary irrigation is given and plants are dependant on rainfall. Since harvesting is done predominantly in November and December, rainy conditions do have a major effect on the quality of the harvest. The rainfall varies from season to season, from a minimum of 60 up to 500mm per annum.

The quality of South African bread wheat cultivars is high as a result of the strict quality grading system used to release cultivars. The accurate evaluation of milling- and baking characteristics (hereafter referred to as quality) of pure-lines and early generation progeny of wheat (*T. aestivum* L.) is therefore of prime importance to breeders, together with high grain yield potential, improved production stability, resistance to disease and pests and desirable agronomic traits (O'Brien and Ronalds, 1983; O'Brien and Panazzo, 1988; Fischer *et al*., 1989).

Differences among wheat cultivars in quality attributes arise from a multitude of genetic factors (Graybosch *et al*., 1996). These quality attributes are not only genetically fixed, but

influenced by both genotype and environment and their interaction (McGuire and McNeal, 1972). This makes the inheritance of wheat quality traits and the efficiency of selection for genotypes possessing outstanding quality characteristics more complex (Barnard *et al*., 2002).

Contributing to the complexity of the inheritance of quality components is the polygenic nature of the characteristics involved in wheat quality (Barnard *et al*., 2002). This has led to the development of a number of quality tests which are used to evaluate the ability of different varieties to satisfy specific requirements of breeders, producers, millers, bakers and consumers (Mullaly and Moss, 1961). Although the aim of breeders is to satisfy the requirements of the market, different testing parameters are of importance to them. Producers, for instance, mainly concentrate on hectolitre mass, falling number and protein content, because these determine the grading and price of their product. Plant breeders, on the other hand, are interested in any parameter that will individually, or in combination with other parameters, give a direct or indirect indication of the overall quality of the specific line. It is essential that this parameter/s should effectively discriminate between genotypes in order to make selection possible without causing a negative effect in a related parameter by indirect selection.

Due to the polygenic nature of bread-making quality, there is an urgent need to supply plant breeders with parameters which give more accurate information about the end-use quality potential of single plants in the early generations of breeding programs. The loss of potentially good genotypes could be decreased by giving the plant breeder an indication of the amount of variation that exists within the population of a specific cross, whereby more informed, more intensive selections in specific combinations with very good quality characteristics can be made. It is this variation that needs to be exploited in the following generations through selection by making single plant and progeny rows or plots selection (Gras and O'Brien, 1992).

The reasoning behind early generation selection is firstly the financial and practical implications. If most undesirable lines can be discarded at an early stage, subsequent costly tests of field plot plantings to evaluate agronomical characteristics, disease resistance, yield, and milling- and baking quality can be avoided (Atkins *et al*., 1965; Gras and O'Brien, 1992). These tests then need only to be conducted on lines that have an increased probability of having acceptable milling- and baking quality (Gras and O'Brien, 1992). If 50% or more early generation material can be discarded by selecting for a single quality character, efficiency of a breeding program might be increased markedly (Lebsock *et al*., 1964).

Secondly, the necessity of identifying genotypes containing desired characteristics in the earliest possible generation is important, for once it is discarded and lost it cannot be retrieved in subsequent generations (Shebeski, 1967). Because of the high number of genotypes that needs to be tested in the early generations of a breeding program, a single quality test giving an indication of the overall quality of the wheat would be ideal to discard only the extremely poor genotypes that can definitely not be considered in future generations. Ideally, this test needs to be cheap, simple and quick to perform.

The discrimination ability of early generation tests has been evaluated in previous studies, but the material involved has usually been from diverse backgrounds, which broadens the genetic variability and may lead to an increase in discrimination ability in the material. It often happens in a breeding program that a certain set of parents was crossed with a specific aim, for instance to improve quality. Although the genetic diversity in the program as a whole would be wide, this would not necessarily be the case in the progeny of this set of parents, because a few donor parents would be used more than once on the best adapted genotypes in the program which needs quality improvement. This situation has led to the initiation of this study.

The objectives of this study were to:

- Assess the effect of genotype x environment interaction on the stability of SDS (sodium dodecyl sulphate) sedimentation in both advanced and early generation material
- Compare the SDS sedimentation and sedimentation value
- Assess the effect of F2 SDS sedimentation selection on the quality of the subsequent F4 generations
- Determine correlations between SDS sedimentation and other characteristics
- Determine the traits responsible for variation in SDS sedimentation
- Determine the optimum allocation of resources, locations, years and replications in a wheat evaluation program evaluating SDS sedimentation

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CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Due to the polygenic nature of bread-making quality, it is necessary to supply plant breeders with more accurate information about the end-use quality potential of single plants in the early generations of breeding programs aiming to release varieties that need to satisfy strict quality grading systems. This will decrease the loss of potentially good genotypes by giving the plant breeder an indication of the amount of variation that exist within the population of a specific cross, whereby more informed, more intensive selection in specific combinations can be made. In comparison with other quality tests, the sodium dodecyl sulphate sedimentation volume test (SDS sedimentation) is a relatively low-cost, less time-consuming test that requires low man-power and no expensive or elaborate laboratory equipment. It has proved to be a reliable, highly reproducible quality parameter that generally gives a good indication of the end-use quality of wheat (Blackman and Gill, 1980; Carter *et al*., 1999), especially in cases where wheat has a low to medium protein content (Krattiger and Law, 1991).

2.2 Early generation selection

There are opposing views on the genetic theory that underlies selection in early generations for quantitative traits in wheat. Although the value of early generation selection is still controversial (Rath *et al*., 1990), it largely depends on the way it is being implemented in the program. It was suggested by Allard (1960) that early generation selection should be restricted to highly heritable traits such as disease resistance and plant height and that traits of low heritability should be evaluated only in the lines that survive this screening. With polygenic traits like yield and quality, additive and non-additive variance are both large in the early segregating generations and the amount of additive and non-additive variance varies between entries, which makes effective identification of promising lines nearly impossible (Allard, 1960). Selection is possible only when a degree of homozygosity is attained and minimal additive or dominance effects are present (in the F5 or F6 generations). In theory, this argument makes sense, since for only a single character; total homozygosity will only be attained in the F7 generation (Table 1). Although selection based on single F2 plants is reported to be effective for simply inherited traits, it has occasionally been found to be ineffective for more complex traits like yield and quality (De Pauw and Shebeski, 1973), thus underlining the view of delayed selection. O`Brien and Ronalds (1987) argued that in the

course of line development, any advanced lines will be subjected to quality evaluation. Had the line exhibited extreme quality variation, it would have been discarded at an early stage. In contrast, random lines in early generations are not selected on quality and did not have time to attain any great degree of homozygosity. Delaying quality selection until breeding lines become more homozygous would probably lead to higher heritability and improved response to selection.

Table 1. Illustration of the homozygosity status of a specific gene (AA and aa) in subsequent generations in a breeding programme

Cross:	Parent AA x	Parent aa
Generation	**Heterozygosity**	**Homozygosity**
F1	100%	0%
F2	50%	50%
F3	25%	75%
F4	12.5%	87.5%
F5	6.25%	93.75%
F6	3.125%	96.875%
F7	1.5625%	98.4375%

The opposing view in favor of early generation selection states that the most desirable gene combinations can be roughly identified even in the heterozygote. The proportion of plants with these combinations decreases rapidly with advancing combinations. The necessity of identifying genotypes containing a desired characteristic in the earliest possible generation is therefore important, for once it is discarded and lost it cannot be retrieved in subsequent generations (Shebeski, 1967; Sneep, 1977; Gras and O'Brien, 1992).

There are many genes with small individual effects that contribute to quality and it seems that the proportion of high quality lines in a population may decrease with each generation where selection for quality is delayed, therefore McNeal *et al*. (1969) suggested that early generation selection for agronomic characteristics should always be accompanied by selection for milling and baking quality to prevent loss of high quality lines.

The practical reasoning behind early generation selection is firstly the financial and time implications. If most lines can be discarded at an early stage, subsequent costly tests of field plot plantings to evaluate agronomical characteristics; disease resistance, yield, and milling- and baking quality can be avoided (Atkins *et al*., 1965; Gras and O'Brien, 1992). These tests then need only to be conducted on lines that have an enhanced probability of having acceptable milling- and baking quality (Gras and O'Brien, 1992). Therefore cost-effectiveness of delayed quality selection in early generations based on the heritability estimates would need to be considered carefully by the breeder before it is adopted (O'Brien and Ronalds, 1987), because of the high numbers in early generations. If 50% or more early generation material can be discarded by selecting a single quality character, efficiency of a breeding program might be increased markedly (Lebsock *et al*., 1964).

Before developing small-scale quality tests, breeders encountered several restrictions, first of all large quantities (±1kg) of seed were required, which were not available until the F6 or F7 generation in a program. Secondly, traditional testing techniques were destructive and there had to be enough seed to make allowance for testing and planting in the following season. Thirdly, many wheat-breeding programs did not have the substantial cereal chemistry support facilities that would have been required to handle the large number of early generation breeding lines. Fourthly, no material was discarded because of poor quality prior to yield testing and information on agronomic performance was gathered prior to quality testing as a matter of policy. Early generation testing is no longer such a limitation and various improved milling-, baking- and other predictive tests have been developed (Gras and O'Brien, 1992) and higher predictability has been accomplished. Rapid and reliable techniques now allow the use of early generation quality tests that require as little as 2g to10g of grain (Brennan and O'Brien, 1991).

The selection procedure has an influence on the success of quality selection (O'Brien and Ronalds, 1984). The sequence in which individual traits are selected depends on their relative importance, ease of selection and interrelationships with other characteristics (O'Brien, 1983). Other major criteria of an early generation bread-making quality test are small sample size, high repeatability, ability to detect genetic variability, strong correlation with functional properties used for final quality assessment, excellent discrimination ability and ability to rank genotypes similarly at different locations and over different seasons of growth (O'Brien and Orth, 1977). The test must also be easy, simple, rapid and preferably with minor financial implications.

Numerous early generation selection studies in wheat breeding programs have been conducted to evaluate the effectivity of these tests with diverse results (Briggs and Shebeski,

1971; Bhatia, 1975; Fischer *et al*., 1989; O'Brien and Panozzo, 1988). The choice of test being implemented is therefore of utmost importance.

The amount of variation that would exist and chance of selecting superior genotypes is restricted not only by the choice of parental genotypes but also by the size of the population in early generations. Shebeski (1967) proved that a minimum of 1330 plant lines would have to be grown in the F2 generation to be able to select only one line that would contain all the desirable alleles for a trait that differs for 25 genes for a polygenic characteristic like yield or quality. Only one plant per 100 000 of plants in the F3 generation will have all the desired genes in either heterozygous or homozygous condition. Accordingly to McGinnis and Shebeski (1968) suggested that a large F3 nursery is necessary to solve this problem.

Wheat varieties with very diverse quality characteristics are frequently used in breeding programs to introduce genes for pest control or special agronomic features (Atkins *et al*., 1965). It makes sense that advance by selection would be higher in populations with higher variability, because the potential to exploit desirable genotypes will be improved.

The large influence of genotype by environment (GxE) interaction that results in low heritability coefficients for most quality parameters is an important aspect to consider in early generation quality selection. The extent of this influence varies between quality parameters and between genotypes (Kadar and Moldovan, 2003). When considering implementation of early generation quality selection in a breeding program, a high correlation between the performance of the genotypes selected in early generations and the performance of their progeny in later generations is essential (Baker *et al*., 1977). Studies determining the heritability of the characteristics that determine wheat quality have been conducted with early generation breeding lines (Pearson *et al*., 1981; O'Brien and Ronalds, 1987). Sunderman *et al*. (1965) reported a significant positive correlation between SDS sedimentation in the F2 and F3 generation. It is argued that the goal of early generation quality evaluation is merely to guide the breeder with early generation selection at a low selection intensity by means of truncation and that a higher selection intensity will only be implemented in the more advanced generations with higher heritability.

Small-scale quality tests used in early generations to do selection have often been criticized, because the results may strongly be affected by external influences and evaluate only certain components of bread-making quality (Lebsock *et al*., 1964). O'Brien and Ronalds (1984) reported that significant correlations between most small-scale and standard measures of flour quality exist, which suggests that small-scale quality tests like SDS sedimentation are reliable (Axford *et al*., 1978) and effectively measure some aspects of flour quality

(presumably related to the endosperm proteins that are associated with determining flour and dough quality) that are not reflected by flour protein content alone. This, however, depends on the specific small-scale test. In South Africa the relative contribution of cultivar, environment and their interaction as sources of variation in quality attributes of advanced material (Van Lill *et al*., 1995a) and the heritability estimates and correlations of bread wheat quality traits (Barnard *et al*., 2002) have been determined, but contributions of these factors to quality in early generation material where segregation still occurs still remains unclear.

Various milling-, baking- and other predictive tests were developed and after the efficiency of these small-scale tests was investigated, breeders started to apply these tests to guide their selection (O'Brien and Ronalds, 1984; Gras and O'Brien, 1992). Correlations are of interest because pleiotropy is a common property of major genes, and it is important to know how the improvement of one character will cause simultaneous changes in the other characteristics and because the relationship between a metric character and fitness is the primary agent that determines the genetic properties of that character in a natural population (Falconer, 1981). Therefore all possible correlations between the different quality and other parameters need to be determined in both early and later generation material to prevent unwanted indirect selection which may lead to a negative influence on other parameters in subsequent generations on other quality aspects. If the correlation is small enough, improvement of one parameter with minimal influence on another would indeed be possible. One of the most important limitations in genetic improvement of wheat quality are firstly negative correlation between yield and quality, which has proved to be small enough that only a small part of protein variation can be explained by variation of yield. This indicates that improvement in protein content, without sacrificing yield, is indeed possible (O'Brien and Panozzo; 1988).

Among all these quality indices, SDS sedimentation has been thought to be one of the more simple and reliable methods (Takata *et al*., 2001) of evaluating the quality potential of a genotype, despite having its limitations (Ayoub *et al*., 1993; Morris *et al*., 2007). Baker *et al.* (1977) reported that protein content alone is not a good indicator of good wheat quality, for high protein values were present in low and high factor groups. SDS sedimentation seems to be an ideal single characteristic because regardless of other quality traits a line may posses, a potential bread variety is not accepted unless its SDS sedimentation is adequate and meets the requirements for protein quality determination in early generation quality selection (Gras and O'Brien, 1992).

2.3 SDS sedimentation test

2.3.1 Background

The need for a single quality test that significantly correlates with known characteristics essential to bread baking that does not give an indication of only one character, but of the mixing and baking worth as a whole, has led to the development of the SDS sedimentation test. Finney and Bains (1999) reported a correlation of r=0.94 between the Zeleny sedimentation test and SDS sedimentation test in soft wheat flours.

The Zeleny sedimentation test was designed by Zeleny (1962 and 1947) and modified by Pickney *et al*. (1957) into its present form. Axford *et al*. (1978) modified the Zeleny test by including sodium dodecyl sulphate (SDS) in addition to lactic acid, while leaving out iso-propanol, which has proved to be superior to the Zeleny test in predicting bread-making quality (Axford *et al*., 1979). The test gives an indication of the physico-chemical behaviour of flour and the protein aggregative ability (Graybosch *et al.,* 1996). By addition of lactic acid to the flour-water suspension, the protein fibrils interact with each other and with flour particles and stability of the particles increases (Krattiger and Law, 1991).

Although the SDS sedimentation test was not initially designed to be used in breeding, it is being used for that purpose because of its simplicity and objectivity and small seed requirement (Greenaway *et al*., 1966). The SDS sedimentation test has been investigated in numerous countries where reports deal in whole or in part with the test (Zeleny, 1947; Pickney *et al*., 1957; Zeleny *et al*., 1960). The SDS sedimentation test was applied on durum (Dexter *et al*., 1980; Quick and Donnelly, 1980; Kovacs, 1985), soft white wheat (Carter *et al*., 1999; Guttieri *et al*., 2004) and hard red winter wheat (Matuz, 1998). Zeleny *et al*. (1960) and Lebsock *et al*. (1964) reported that the SDS sedimentation test might have value when used in conjunction with other wheat quality tests.

Studies utilizing European wheat covering a wide range of bread-making quality proved that the SDS sedimentation test was superior to either the Pelshenke dough-ball or Zeleny sedimentation tests in predicting loaf volumes of bread produced by Chorleywood and fermentation procedures (Axford *et al*., 1978; 1979). Similar results were obtained by Blackman and Gill (1980).

There has been evidence to indicate that the SDS sedimentation test singularly gives the best prediction of bread baking potential and strength for hard wheat (Greenaway *et al*., 1966; Moonen *et al*., 1982). Fowler and de la Roche (1975a) confirmed this by reporting that sedimentation values accurately reflect quantity of protein and rate of dough development, both of which are basic quality measurements.

2.3.2 Physico-chemical basis

The SDS sedimentation test basically measures the sedimentation volume of an acidified suspension of wheat flour. The higher the sedimentation volume, the better the baking quality (Eckert *et al*., 1993).

Frazier (1971) did a comprehensive study of the physico-chemical mechanism (particle-liquid) and particle-particle interactions that influence sediment of the Zeleny test. Krattiger and Law (1991) gave a comprehensive overview of the biochemical basis of the SDS sedimentation test. Adeyemi and Muller (1975) reported that SDS sedimentation volumes were found to be primarily dependent on flocculation and it was concluded that differences in sediment volume between flours were due to the proportion of the flocculating particles in the flour and not, as previously supposed, to glutenin swelling. In contrast to this, Eckert *et al*. (1993) reported that the sediment in the SDS sedimentation solution theoretically results from the swelling of the glutenin strands and the interaction of starch and gluten (Carver and Rayburn, 1995). It has long been known that differences between flour from different types of wheat are reflected in the ability of the gluten protein to imbibe water, because gluten swells in dilute lactic acid (Krattiger and Law, 1991). Investigation of gliadin and glutenin showed only glutenin to be capable of swelling, whereas gliadin dissolved completely (Eckert *et al*., 1993). It thus appears that the mechanism responsible for gluten swelling is glutenin related (Sapirstein and Suchy, 1999).

Detergents, such as SDS bind to protein molecules creating a negative charge, which is independent of the charge on protein, although proportional to its molecular size. In aqueous solutions, these colloidal complexes create a diffuse electrical double layer. Since all colloidal particles possess a negative charge, they are repulsive. By adding acid, these repulsive charges are neutralized and so the colloidal particles associate to create larger flocks and sediment is formed (Krattiger and Law, 1991).

2.3.3 Effectivity

The SDS sedimentation test is superior to the Zeleny test in predicting bread-making quality as determined by the Chorleywood bread baking process (Blackman and Gill, 1980). In the past few years the SDS sedimentation test has been investigated on different wheat types in numerous studies and countries (Axford *et al*., 1979; O`Brien and Ronalds, 1987; Matuz, 1998, Takata *et al*., 2001; Morris *et al*., 2007). It has gained wide acceptance as a useful, small-scale test in bread wheat breeding programs to give a good indication of differences in both protein content and gluten quality, the two most important factors influencing bread-baking quality (Axford *et al*., 1978; De Villiers and Laubscher, 1995; Carter *et al*., 1999),

especially in genotypes with a protein content up to approximately 13%, where high SDS sedimentation volume have been associated with stronger gluten and good quality (Carter *et al*., 1999). The regression of viscosity on protein content is different for each variety (Zeleny, 1947) and regression of the slope is found to reflect protein quality.

One disadvantage in the standard sedimentation test for breeding worth is that the maximum sedimentation value attainable is not sufficiently high to reflect the true quality of very strong wheat at high protein content. In the case of evaluating genotypes with protein content levels higher than 13%, the SDS sedimentation test seems to be ineffective according to Ayoub *et al*. (1993) who investigated SDS sedimentation volume as a differentiating tool for eastern Canadian wheat of different remix loaf volumes. This is in accordance with the findings of Preston *et al*. (1982). According to Greenaway *et al*. (1966), the SDS sedimentation test might provide useful supplemental information to assist in classifying breeding lines into broad quality ranges, but does not give an indication of loaf volume in commercial lots. This "ceiling effect" is much more troublesome with breeders` samples than with commercial wheat, since the experimental plots used by the breeders are often highly fertilized, resulting in wheat of very high protein content and consequently often very high sedimentation volume (Greenaway *et al*., 1966).

2.3.4 Discrimination ability

2.3.4.1 Durum

Baik *et al*. (1994), Dexter *et al*. (1980), Quick and Donnelly (1980) and Cubadda *et al*. (2007) investigated the suitability of SDS sedimentation as an indication of pasta quality of durum wheat. Quick and Donelly (1980) and Dexter *et al*. (1980) stated that the SDS sedimentation test in combination with wheat protein, is adequate to screen durum wheat for gluten strength and spaghetti cooking quality. Baik *et al*. (1994) found SDS sedimentation suitable for screening the quality of Oriental noodles as related to protein content and protein quality of a flour.

2.3.4.2 Soft white wheat

Carter *et al*. (1999) found that the SDS sedimentation test is an effective small-scale test for end-use quality assessment in soft white and club wheat breeding programs.

2.3.4.3 Hard red wheat

SDS sedimentation tests have been performed on winter wheat (Miller *et al*., 1956; Barmore and Fifield, 1964). Preston *et al*. (1982) applied the test to Canadian bread wheat and found

that the sample weight had to be reduced from 6.0 to 4.5g and the final swelling time from 20 to 15min, since the strong, high-protein Canadian Western Red Spring wheat gave sedimentation volumes too large for satisfactory discrimination. This was confirmed by Ayoub *et al.* (1993), who reported that SDS sedimentation was unable to differentiate between the eastern Canadian bread wheat, especially if they had high protein content.

Lorenzo and Kronstad (1987) found that SDS sedimentation is most effective to discriminate between winter wheats' materials from Oregon State University when using 5g samples, suspended in 100ml of solution containing 0.96g lactate l^{-1} and 20g SDS l^{-1} and a reading time of 30min, although dependent upon variations in protein content. Although Moonen *et al*. (1982) found a correlation between the SDS sedimentation and bread volume ($r=0.87$; $P\leq0.001$), SDS sedimentation was unable to discriminate between bread-making quality subgroups. In contrast to this, Briggs and Shebeski (1971) found an inconsistent predictive ability of SDS sedimentation in the early generations of hard red spring wheat.

2.3.5 Infuencing factors

2.3.5.1 Chemicals

The SDS sedimentation test has been modified to make it applicable to soft as well as hard wheat, and to provide greater uniformity between laboratories (Pickney *et al*., 1957). Several modifications have been made to the procedures of the initial SDS sedimentation test to increase the correlation of this test with bread-baking quality (Preston *et al*., 1982; Lorenzo and Kronstad, 1987; Krattiger and Law, 1991) and to eliminate its dependence on protein content (Krattiger and Law, 1991).

In the traditional method, flour (5g) or whole meal (6g) were suspended in water (50ml) by rapid shaking for 15sec in 100ml stopper measuring cylinders and similarly shaken at 2 and 4min. Two percent SDS (50ml) was added and the contents of the cylinders mixed by inverting four times. The cylinders were similarly inverted at three further time intervals of 2min and 1:8 v/v 85% lactic acid:water (1.0ml) was added and the contents again equilibrated as in the case of the SDS addition. Finally, the contents were allowed to settle for a period of 40min (flours) or 20min (whole meals) and the volumes of the sediments recorded. There is evidence that the SDS and lactic acid may be added simultaneously (Axford *et al*., 1978).

The SDS sedimentation test has been criticized for being complicated by the need for two or more reagent solutions, and seemingly arbitrary time schedules for shaking the suspension, inverting and resting the measurement cylinders (Sapirstein and Suchy, 1999). Greenaway

et al. (1966) modified the SDS sedimentation test by increasing the amount of lactic acid reagent in order to increase the volume of liquid in the cylinder and thus eliminating the "ceiling effect" of the standard test. The spacing in the millroll was also increased in order to increase flour yield and the wheat moisture content was adjusted to about 13% prior to milling to eliminate variable moisture content. The concentration of the lactic acid and isopropyl alcohol in the lactic acid reagent are adjusted accordingly so that, after mixing with the hydration water, the concentrations in the final mixture are the same as for the standard test (Greenaway *et al*., 1966). Dexter *et al*. (1980) used 2% SDS and 5g ground grains and found that by altering the SDS concentration it was possible to change the range of SDS sedimentation volume values without affecting the ranking of samples. O'Brien and Ronalds (1983) simplified the SDS sedimentation procedure to make it possible to be used for quality testing in early generations of a wheat breeding program.

Dick and Quick (1983) reduced the wholemeal wheat sample and varied other parameters, including the SDS concentration in durum. McDonald (1985) used a higher concentration SDS to better differentiate between strong and weak gluten durum wheat than does the original procedure. The SDS concentration was slightly reduced to 4.7g l^{-1} by McDonald (1985), which then became the AACC Approved Method 56-70 for durum wheat. Kovacs (1985) optimized the SDS sedimentation test by increasing the hydration time to 12 min and using 3% SDS solution containing 0.8% lactic acid. While maintaining the sedimentation time at 20 min, the numerous shaking steps of the original method were eliminated in order to analyze more samples per day. Lorenzo and Kronstad (1987) tested different combinations of SDS, lactate solution and reading times to improve the correlation between loaf volume and SDS sedimentation.

2.3.5.2 Sample size

The efficiency of reducing sample size of the initial SDS sedimentation test has also been investigated by several authors (DeWey, 1963; Wise *et al*., 1965; Dick and Quick, 1983, Evlice *et al*., 2007). Evlice *et al*. (2007) reported correlations of r=0.81 to 0.96 between the standard SDS sedimentation test and the mini SDS sedimentation test using a smaller sample of grain.

Reducing sample size results in the reduction of SDS sedimentation volume. This reduction is consistent, though, resulting in no differences in ranking (DeWey, 1963). In contrast to this, Carter *et al.* (1999) found that changes in protein concentration and sample weight caused proportional changes in SDS sedimentation volumes, but that the response was not consistent among different genotypes. Sample size was positively correlated with SDS sedimentation volume for whole meal (r=0.51) and flour (r=0.61) at $P \leq 0.05$, but the

magnitude of the effect of sample size depends on environment and genotype (Carter *et al*., 1999). It was reported that sample weight caused proportional changes in SDS sedimentation volumes in soft white wheat, but it did not influence the discrimination ability of the test (Carter *et al*., 1999).

It would seem from the literature that sample weight could be adjusted for individual convenience (Carter *et al*., 1999), because the reproducibility seems to be good with smaller samples too, but the sample weight must be measured very accurately within a trial to eliminate the significant effect of varying sample weight.

Originally the test was designed to use 200g of seed, but Zeleny *et al.* (1960) and Wise *et al*. (1965) proved that 2-5g could evaluate wheat satisfactorily, thus enabling the breeder to evaluate the quality of early generations. DeWey (1963) reported that samples of 40g or slightly less can be effectively used for a standard test and proved to be useful to breeders. It is, however, consistently lower in value, but gives a good indication of the relative ranking of the standard 220g samples, although varieties might be differently affected by size of the sample. Wise *et al*. (1965) developed a micro modified technique in response to a request by Idaho wheat breeders for a quality test on 5g samples of wheat selections. Through this method, an index of quality characteristics may be determined in the F2 generation of wheat breeding – much earlier than the standard 25g.

Dick and Quick (1983) reviewed the effectiveness of a micro SDS sedimentation test to give an indication of early generation durum wheat breeding lines used for spaghetti pasta. The 1g micro test was superior to the standard SDS sedimentation test to indicate spaghetti firmness.

Matuz *et al.* (1986) investigated the efficiency of a small-scale SDS sedimentation test requiring only 0.5g of flour. This test has a lower labour requirement, because instead of manual shaking (Matuz and Medovarszky, 1986), samples are mixed by a “sedimator” machine, which was developed by Lelley (1973). It has been criticized for other practical difficulties, though, because of dough getting stuck in the tubes (Chrissie Miles, personal communication).

Lorenzo and Kronstad (1987) used five hexaploid varietal wheat samples to evaluate the effects of SDS concentration, lactic acid concentration and settling time and found that SDS sedimentation is most effective to discriminate between winter wheat materials from Oregon State University when using 5g samples, suspended in 100ml of solution containing 0.96g

lactate l^{-1} and 20g l^{-1} SDS and a reading time of 30min, although it depended upon variations in protein content.

Krattiger and Law (1991) reported a correlation coefficient between loaf volume and SDS sedimentation of r= 0.82, when using a sample size of 5g and r=0.78, when using a sample size of 1.5g of flour (P≤0.001), thus only a small reduction. No time-dependent interaction effects on SDS sedimentation volume existed. Small changes in SDS concentration and reduction in sample weight also had no effect. Krattiger and Law (1991) reported that addition of 0.2% 2-mercaptoethanol or sodium chloride improved correlation coefficients between SDS sedimentation and quality characteristics with the increase in correlation depending on the time of recording. The effect of sodium chloride must be due to the reduction in the electrostatic repulsion of the negatively charged SDS-protein complex (the higher volume), and also interaction with damaged starch (Krattiger and Law, 1991). Centrifugation at 1000xg max after performing the SDS sedimentation test also improves the correlation with quality, while correlation with protein content and grain hardness is reduced to non-significance. When adding 0.2% 2-mercaptoethanol, there was no significant correlation between SDS sedimentation and loaf volume, but the effect of protein content on SDS sedimentation volume was reduced. The correlation between SDS sedimentation and protein content was also reduced when adding sodium chloride (Krattiger and Law, 1991).

Baik *et al.* (1994) modified the SDS sedimentation test by including nine sediment volume readings, three each after three mixing times. Carter *et al*. (1999) examined the use of a modified micro sedimentation test for soft white and club wheat. Sediment volumes were highly dependent on sample weight (0.35 to 0.80g) and the response to weight varied among varieties and protein concentrations. Different sample weights and substituting whole meal for flour did not affect the ability of SDS sedimentation test to differentiate among lines, however (Carter *et al*., 1999).

SDS sedimentation generally increases with SDS concentration until reaching a threshold concentration of 10 to 15gl^{-1} (Dick and Quick, 1983; Kovacs, 1985; McDonald, 1985; Lorenzo and Kronstad, 1987). Since the response is due to inherent differences among wheat samples, it is not consistent between varieties (Morris *et al*., 2007). The primary consideration appears to be to supply sufficient SDS to accommodate the protein/strong-gluten samples.

2.3.5.3 Whole meal vs. white flour

Both whole meal and flour can be used to perform the SDS sedimentation test, but the bran in whole meal may reduce the effectiveness and accuracy of the test. McDermott and

Redman (1977) found that the correlation between whole meal SDS sedimentation and loaf volume were higher (r=0.93) than that of the white flour SDS sedimentation and loaf volume (r=0.81). In contrast to this, Axford *et al*. (1979) and Carter *et al*. (1999) reported that substituting whole meal for flour did not affect the ability of the SDS sedimentation test to differentiate among lines. Dick and Quick (1983) and Kovacs (1985) also reported that whole meal (which is more convenient to prepare) performs similar to milled flours.

2.3.5.4 Settling time

Morris *et al*. (2007) reported that the rate of sedimentation in a solution of sodium dodecyl sulphate and lactic acid is fairly rapid, such that most settling has already taken place within 5min and little further settling occurs by 10min. The rate of settling during this early time (0 to 5min) is actually inversely related to the final sediment volume, i.e. the rate for Hiller Club wheat was by far the greatest because it reached its lowest volume before 5min had elapsed. After 10min there was essentially no change in ranking of samples for sedimentation volume. Optical monitoring devices could likely discern sedimentation velocity differences among samples before the eye could delineate a demarcation between supernatant and sediment. Recording time can be a large source of variation, due to the differences in settling of the sediment over time, but interactions are relatively small, though, so that a recording time of at least 10min or more consistently ranked samples (Morris *et al*, 2007).

2.3.5.5 Time interval between grinding and testing

DeWey (1963) reported that no apparent change in sedimentation value for different genotypes occurred if the sample is ground and only tested from 1 to 7 days after grinding, material could therefore be ground the day before testing. McDonald (1985) reported that grinding rate has an effect on sedimentation volume. Slower grinding rates produced higher sedimentation volumes, possibly due to differences in particle size distribution.

2.3.5.6 Carry-over effect in rolls

Although the specific grinder type has a minor influence on SDS sedimentation (Morris *et al*., 2007), the carry-over effect from one SDS sedimentation sample to the next when the grinder rolls were not cleaned between samples, could be of particular concern to a plant breeder who was consecutively testing selected material differing widely in SDS sedimentation values if samples smaller than 200g are used (DeWey, 1963).

2.3.5.7 Protein

It is important to separate the two major factors contributing to a large portion of variation in quality differences in wheat cultivars, namely protein quality (composition) and quantity

(concentration) (Bushuk *et al*., 1969; O'Brien and Panozzo, 1988; De Villiers and Laubscher, 1995; Carter *et al*., 1999; Graybosch *et al*., 1996). The protein concentration is genetically determined, but strongly influenced by environmental factors (DuPont and Altenbach, 2003; Wieser and Seilmeier, 1998; Payne, 1987). The composition of the proteins and protein subunits is genetically fixed (O'Brien and Ronalds, 1983). It is therefore commonly accepted by cereal chemists that protein quality under different growing conditions is almost entirely an inherited characteristic (Bushuk *et al*., 1969, DuPont *et al*., 2007). However, the relative quantity of specific proteins, protein subunits, protein groups and amount of polymeric proteins varies due to environment and genetic determination (Wieser and Seilmeier, 1998).

Environment-dependent changes in protein quantity influence SDS sedimentation volume, not only because of high protein content resulting in higher absolute glutenin amounts and higher glutenin:gliadin ratios (Johansson *et al*., 2001), but also due to changes in protein-starch interaction (Graybosch *et al*., 1996). Ozturk and Aydin (2004) reported that an increased SDS sedimentation due to water deficit can be explained by an increase in grain protein mainly due to higher rates of grain nitrogen accumulation and lower rates of carbohydrate accumulation.

Several studies investigated the influence of environmental factors on flour protein content and composition (Baenziger *et a*l., 1985; Johnson *et al.* 1972; Van Lill *et al*., 1995 a and b ; DuPont *et al*., 2007). Since it has been well established that the SDS sedimentation test accurately asseses protein quality (Dick and Quick, 1983; Preston *et al*., 1982; Carter *et al*., 1999; Saint Pierre *et al*., 2008), specifically the ratio of various gluten proteins (Zhang *et al*., 2008) and protein concentration (Dick and Quick, 1983; Preston *et al*., 1982; Saint Pierre *et al*., 2008), the significant influence of the environment on SDS sedimentation is confounded by the large effect of protein on SDS sedimentation volume (Carter *et al*., 1999; Lorenzo and Kronstad, 1987; De Villiers and Laubscher, 1995; Van Lill *et al*., 1995b). Therefore any factor changing protein quantity and proportion will inevitably have an influence on SDS sedimentation.

Several environmental conditions influencing SDS sedimentation (because of the influence on protein) have been reported (Blumenthal *et al*., 1993; Saint Pierre *et al*, 2008). Temperature, humidity (Graybosch *et al*., 1996), moisture availability (Saint Pierre *et al*., 2008), nitrogen availability (Wieser and Seilmeier, 1998), planting density (Matuz, 1998) have been reported as such factors.

It is well known that nitrogen application influences protein quantities and proportions (Johansson *et al*., 2001; Wieser and Seilmeier, 1998), therefore it will inevitably have an

influence on SDS sedimentation volume. The increase in SDS sedimentation with nitrogen application is higher under water stress than under well-watered conditions (Saint Pierre *et al*., 2008). According to Gooding *et al*. (2003), SDS sedimentation is more sensitive to drought stress than temperature stress. Decline in SDS sedimentation at high temperatures and low relative humidity is a commonly observed effect, especially if the temperatures and humidity are less than 40% respectively. Optimal SDS sedimentation was observed with exposure to temperatures higher than 32ºC for less than 90 hours (Graybosch *et al*., 1996). Planting density affects SDS sedimentation, but the ranking in different trials was similar (Matuz, 1998).

Gluten

Wrigley and Bietz (1988) gave an overview of the composition and molecular characteristics of gluten. High protein does not necessarily indicate high gluten content and high gluten does not necessarily indicate high quality (Wang *et al*., 2004). It is both the quantity and structure of gluten proteins that determine dough properties and baking performance and this is strongly dependent on genotype and growing conditions (Wieser and Seilmeier, 1998). Protein constitutes a complex mixture of many different protein components of which mainly two types, water-insoluble gluten constituents occur, namely gliadin and glutenin (O'Brien and Panozzo, 1988; Graybosch *et al*., 1996; Carter *et al*., 1999). Gliadin subunits are monomeric single chain polypeptides of similar molecular weight forming only intra-chain disulfide bonds, while glutenin subunits are polymeric forming intra- and inter-chain disulfide bonds (Rakszegi *et al*., 2005). Graybosch *et al*. (1996) reported that glutenin is the major protein determining dough characteristics and is nearly totally genotype dependent.

Graybosch *et al*. (1996) reported that protein quality (composition of protein fractions) is more susceptible to environmental modification than flour protein content. Even if the gluten contents of all the cultivars may be the same, these may not be in a linear relationship with their processing quality, because of differences in composition (Zhang *et al*., 2008).

It is important to distinguish between quality and quantity when referring to gluten, since these two characteristics exist independently (Zhang *et al*., 2008). The roles of both are accentuated by the fact that gluten generally increases on a total-protein basis as protein content increases (Wrigley and Bietz, 1988). Zhang *et al*. (2008) reported that the ratio of the Zeleny sedimentation value to dry gluten can reflect the quality of the gluten and that this ratio may more objectively differentiate between the gluten quality between varieties. Soft white and club wheat should have low SDS sedimentation volumes due to their weak gluten (Zhang *et al*., 2008).

With knowledge of gluten composition increasing, researchers have attempted to relate differences in gluten composition to dough properties. Gliadin increase results in decrease in mixing time, peak resistance, maximum resistance to extension and loaf height. An increase in gliadin would result in an increase in resistance to breakdown and extensibility (Uthayakumaran *et al*., 2001).

With knowledge of gluten composition increasing, researchers have attempted to determine the effect of different environment influences on gluten composition. Under certain stress conditions, a change in SDS sedimentation due to changes in protein proportion could be attributed to the gluten composition, with an increase in gliadin (monomeric) opposed to glutenin (polymeric), because the synthesis of some monomeric proteins is increased (Blumenthal *et al*. 1993; Graybosch *et al*., 1996; Saint Pierre *et al*., 2008). Nitrogen supply causes an increase in gliadin and glutenin, but not albumin and globulin (Johansson *et al*., 2001). Graybosch *et al.* (1996) reported that the production of flour non-gluten protein was elevated with increased temperatures during grain filling, while gluten (both gliadin and glutenin) decreased, explaining the decrease in SDS sedimentation.

High and low molecular weight glutenin subunits

The variations of gluten composition have been reviewed by Payne (1987). There are about 20 subunits of glutenin, which differ in their effect on wheat protein quality and are therefore very complex (Kadar and Moldovan, 2003). There are two major types of glutenin subunits, high molecular weight glutenin subunits (HMW-GS) and low molecular weight glutenin subunits (LMW-GS). Both influence quality but the magnitude of the individual alleles varies substantially (Rakszegi *et al*., 2005). The amount of glutenin polymer and type of HMW-GS and LMW-GS that form the polymer mainly determine quality (Payne, 1987). Although hexaploid wheat has six HMW subunit genes, only three to five of them are expressed. Each subunit accounts for about 2% of the total grain protein, so different gene expression results in different amounts of HMW-GS (Rakszegi *et al*., 2005).

Bread wheat has six genes for HMW-GS, encoding closely linked x and y subunits on the long arms of chromosome 1A, 1B and 1D (DuPont *et al*., 2007). HMW-GS Dx5 and Dy10 are correlated with high baking quality and 2 and 12 with poor quality (Payne, 1987). Allelic variation at the *Glu-1* locus, containing genes encoding HMW-GS, has been shown to largely contribute to differences in bread-making quality (Payne, 1978). The genes at loci 6 (Glu-1 and Glu-3 genes) are responsible for these polymeric proteins (Eagles *et al*., 2002). Gupta and Shepherd (1990) reported that genes coding for HMW subunits of glutenin are located at Glu-A1, Glu-B1 and Glu-D1 on the long arms of chromosome 1A, 1B and 1D, while LMW

glutenin subunit loci Glu-A3, Glu-B3 and Glu-D3 are on the short arms of the same chromosomes.

Attempts have been made to link the different HMW-GS to various measures of quality as (reviewed by Wrigley and Bietz, 1988). HMW-GS have a qualitative and quantitive effect on quality (Rakszegi *et al*., 2005). The variation and structure of HMW-GS correlate strongly with dough strength, while LMW-GS and gliadin composition affects dough extensibility (Uthayakumaran *et al*., 2001). Glu-D1 HMW-GS (Dx5+Dy10) make a larger contribution to dough properties than Glu-B1 (Bx17+18), with Glu-A1 (Ax1) making the smallest contribution (Rakszegi *et al*., 2005).

Significant relationships between SDS sedimentation and Glu-1 (Carillo *et al*., 1990) and SDS sedimentation and Glu-3 (Gupta and MacRitchie, 1994) were reported. The Zeleny sedimentation volume markedly increased when HMW-GS (Ax1) was present (Zhang *et al*., 2008). Glutenin composition at Glu-D1 was correlated with both SDS sedimentation and mixograph peak time performance. Payne (1987) suggested that Glu-3 rather than Glu-1 loci are the primary determinant of differences in SDS volumes.

Several studies have focussed on HMW-GS (Dx5+Dy10), often present in strong dough and HMW-GS (Dx2+Dy12) associated with dough weakness. This was confirmed by Saint Pierre *et al*. (2008), where in most of the genotypes, subunits Dx5+Dy10 have been associated with a high SDS sedimentation and higher dough strength than subunits Dx2+Dy12 at the Glu-D1 locus. Subunits GluD1 (5+10) are associated with higher SDS sedimentation and dough strength opposed to subunits GluD1 (Dx2+Dy12).

Genes encoding HMW-GS result in an increase in dough strength (Rakszegi *et al*., 2005). It was concluded that glutenin subunits could be used as markers for selection of genotypes with superior dough properties (Saint Pierre *et al*., 2008).

Carter *et al*. (1999) reported that, since the sediment in the SDS solution theoretically results from the swelling of the glutenin strands in the gluten, cultivars with different protein quality, as expressed by their gluten characteristics, should successfully be differentiated by the SDS sedimentation test.

There are different suggestions as to how and to what extent environmental factors influence the proportions of the individual HMW-GS subunits (DuPont *et al*., 2007). Nitrogen availability and temperature during grain filling influences the different protein types in the

flour, the ratio of HMW-GS to LMW-GS, the amount of HMW-GS per grain, and the proportion of HMW-GS per unit of flour protein (DuPont *et al.,* 2006).

Wieser and Zimmermann (2000) concluded that there is little variation in proportion of HMW-GS for genotypes with the same subunit combination and that growing conditions have little effect on the proportions. DuPont *et al.* (2007) reported that although the amounts of HMW-GS per unit of flour are strongly affected by the environment, the different subunits respond so similarly to external conditions that their final proportions appear to be determined mainly by genetic factors. This was confirmed by Saint Pierre *et al*. (2008) reporting that genotypes of similar protein quality and composition responded similarly to nitrogen application and stress treatments.

Carceller and Aussenac (2001) proposed that the ratio of x to y-type HMW-GS increased during grain fill, indicating that this ratio might be susceptible to environmental effects. Nitrogen application increased hydrophilic protein types (ω-gliadins, HMW subunits of glutenin) and decreased hydrophobic proteins (γ-gliadins and LMW subunits of glutenin), but the degree of the effects on both quantity and proportions of flour protein and gluten protein types was strongly dependent on genotype (Wieser and Seilmeier, 1998).

Since the SDS sedimentation test reflects differences in both protein content and gluten quality, it is particularly useful for wheat evaluation when large differences in gluten quality are prevalent (Pickney *et al*., 1957), but markers in glutenin-subunits allowing selection could improve effectivity in the future.

2.3.5.8 Environment

Bread-making quality traits linked with protein content and gluten characteristics are complex heritable traits, and open to genetic improvement, but strongly influenced by environment (soil-, climatic- and production conditions) (Kadar and Moldovan, 2003). The various components of flour protein differ in their response to environmental and genotypic influence and will change according to the location, cultivating conditions and season even though the protein or gluten quantity are the same for all the cultivars. Furthermore, the proportion of various proteins e.g., the ratio of glutenins to gliadins, will vary (Zhang *et al*., 2008).

SDS sedimentation is not as greatly influenced by the environment, as protein content. It is therefore suggested that indirect selection for protein content via SDS sedimentation would result in effective improvement in protein content of F3 lines (Sunderman *et al*., 1965). It is well known that SDS sedimentation is influenced by protein concentration and the magnitude of the effect varies according to the genotype (Moonen *et al*., 1982; Dick and Quick, 1983;

Lorenzo and Kronstad, 1987; De Villiers and Laubscher, 1995; Carter *et al*., 1999; Gooding, 2003, Cubadda *et al*., 2007; Morris *et al*., 2007). This was confirmed by Carter *et al*. (1999) who reported that protein content has a proportional influence on the SDS sedimentation of soft white wheat, but that the response differed amongst lines.

Kadar and Moldovan (2003) reported that the factor contributing the most to the variation in sedimentation volume is genotype (89.98%), followed by GxE interaction (9.73%) and environment (0.28%). The broad sense heritability was 0.89. To conclude, SDS sedimentation is influenced by environment, crop year, and their interactions with cultivar or specific genotype (Graybosch *et al*., 1996; Carter *et al*., 1999). Nevertheless the test is highly heritable and can be used for selecting amongst early generation progeny (Matuz, 1998).

Atkins *et al*. (1965) stressed the importance of testing lines that are to be evaluated for quality under optimum cultivation conditions which do not limit the expression of genetic potential for these characters, because cultivation under severe nitrogen deficiency leads to sedimentation and other quality values with such a narrow range that they can be unsatisfactory for evaluation of genetic differences among lines. Genotypic differences in both protein content and SDS sedimentation were more easily detected in the less stressed environments (Graybosch *et al*., 1996).

2.3.6 Correlations

2.3.6.1 Loaf volume

High SDS sedimentation has been associated with superior bread-baking quality (Axford *et al*., 1979; Blackman and Gill, 1980; Dexter *et al*., 1980; Preston *et al*., 1982; Dick and Quick, 1983; O'Brien, 1983; Lorenzo and Kronstad, 1987; Ayoub *et al*., 1993). A high correlation between SDS sedimentation and specific loaf volume or loaf score was found in previous studies (Pickney *et al*., 1957; McDermott and Redman, 1977; Axford *et al*.; 1978; Blackman and Gill, 1980; Dexter *et al*., 1980; Moonen *et al*., 1982; Lorenzo and Kronstad, 1987; Krattiger and Law, 1991; De Villiers and Laubscher, 1995). However, the environment plays an important role in the ability of the SDS sedimentation to predict loaf volume, with the major environmental factor being protein content (Preston *et al*., 1982; Carter *et al*., 1999). This was confirmed by De Villiers and Laubscher (1995) and Van Lill *et al*. (1995 a; b) under South African conditions and by Fischer *et al.* (1989) with segregating material. Therefore SDS sedimentation should not be interpreted as a measurement of loaf volume unless the corresponding protein content values are taken into consideration (Lorenzo and Kronstad,

1987). Nitrogen availability also has an influence on the correlation between the SDS sedimentation and bread volume (De Villiers and Laubscher, 1995; DeWey, 1963).

Correlation coefficients found by several authors vary between r=0.86 and r=-0.21, with lower correlations when segregating lines are evaluated (Ayoub *et al*., 1993; Krattiger and Law, 1991; Preston *et al*., 1982; Moonen *et al*., 1982; Fowler and de la Roche, 1975b; Pickney *et al.,* 1957, Atkins *et al*., 1965; Sunderman *et al*., 1965, Zeleny 1947). It is clear that correlations also differ between cultivars. In three of the cultivars, correlations improved when calculated independently from the protein content, suggesting that the latter has an influence on the sedimentation value. Sapirstein and Suchy (1999), found a correlation of r=0.97 and 0.94 between SDS sedimentation and loaf volume for ground wheat and sieved ground wheat, respectively. The lower values for the ground wheat samples could be attributed to removal of coarse bran material by sieving, thereby increasing the concentration of endosperm and constituent gluten protein, on which the nature of the SDS sedimentation test depends. The reduction in average particle size and possible classification of gluten proteins by sieving may also be contributing factors. Therefore, in most of the studies, good correlation coefficients were seen between SDS sedimentation volume and loaf volume (Matuz, 1998).

2.3.6.2 Protein content and quantity

Under South African conditions it was reported that environmental effects dominate variation in protein content and bread volume in spring wheat cultivars in the Western and Southern Cape and large variation exists for characteristics such as protein content and baking strength index amongst winter wheat cultivars grown in the Free State (Van Lill *et al*., 1995a). In accordance, Van Lill *et al.* (1995b) reported that climatic conditions during grain filling significantly influenced grain protein content. It is thus well known that protein content is strongly influenced by the environment (Kadar and Moldovan, 2003), indicating that environment x line interactions could reduce effectiveness of early generation selection for quality characteristics under certain conditions (Lebsock *et al*., 1964).

The large environmental influence on protein content explains the influence on related parameters like SDS sedimentation (Baenziger *et al*., 1985; Fowler and de la Roche, 1975c; Van Lill *et al*., 1995a) and the relationship between these two tests vary depending on season and location. De Villiers and Laubscher (1995) reported that different correlation coefficients existed between the SDS sedimentation and protein content at different locations and Bushuk *et al*. (1969) reported that correlation between flour protein and SDS sedimentation varied in two different seasons (r=0.27 and 0.93).

Preston *et al*. (1982) and Lorenzo and Kronstand (1987) reported that with low protein contents (less than 13%), a high correlation coefficient between protein content and SDS sedimentation was evident, whereas when protein contents were above 14%, the relationship was significantly negative or non-significant. Because the lowest protein content samples give the highest correlation coefficients between SDS sedimentation and loaf volume, the SDS sedimentation should be a very useful screening test under these conditions. Ayoub *et al*. (1993) confirmed this by reporting that sedimentation value was unable to differentiate between the eastern Canadian bread wheat, especially if they had high protein content. Atkins *et al*. (1965) reported a correlation between protein content and sedimentation value of r=0.58 in the F5, r=0.56 in the F6 and r=0.76 in the F7 ($P \leq 0.01$).

Sunderman *et al*. (1965) reported a correlation between SDS sedimentation and protein content in both the F2 (r=0.383) and F3 generation (r=0.638) ($P \leq 0.01$). By selecting high SDS sedimentation in the F2 generation, lines with high protein contents in the F3 were effectively selected. Since the relative sedimentation values were less influenced by the environment than protein content, more improvement in the protein content of F3 lines would have been made by taking advantage of the association of protein with sedimentation value than by direct selection.

Fowler and de la Roche (1975b), Krattiger and Law (1991) and Finney and Bains (1999) encountered relatively high correlation coefficients between sdssed and protein content. Fowler and de la Roche (1975b) reported a correlation of 0.76 ($P \leq 0.01$) between protein content and SDS sedimentation. Dexter *et al*. (1980) reported a correlation of -0.28 ($P \leq 0.01$) of SDS sedimentation and wheat protein in durum wheat. Preston *et al*. (1982) reported a correlation of 0.63 and 0.74 between SDS sedimentation and flour protein for group 1 and 2 respectively ($P \leq 0.05$), where the first group was chosen from commercially grown Canadian wheat of various classes and grades and the second group was chosen to represent the range of grades and protein contents normally encountered in samples of commercially grown Canadian hard red spring wheat. Considering the wide range of flour protein content of the samples in the first group, the lower correlation is not surprising, but only suggests that SDS sedimentation measures protein quality rather than difference in protein content. This is in accordance with reports by Blackman and Gill (1980).

Kitterman and Barmore (1969) found that many samples with similar sedimentation values had considerably different protein contents in both soft common types and soft club types. They stated that for any sedimentation values above 10.5cm (with their method), no material can be distinguished on sedimentation or protein content individually and that both these test are needed.

Lower relative SDS sedimentation was detected when protein content increased above 14%. The concentration of SDS in the test may not be enough to dissolve the higher amount of protein present in the flour sample and the additional protein synthesised at the higher protein levels may not contribute to the viscoelastic properties of the dough, resulting in lower SDS sedimentation values (Lorenzo and Kronstad, 1987). With such flours, the fibrillar particles might be larger and, because the amount of SDS that is bound to the protein in the SDS-lactic acid solution depends on the circumference of the protein complex, the repulsion force between particles would decrease and hence the sedimentation rate would be lower for flours of high protein content (Krattiger and Law, 1991).

In contradiction with previously mentioned literature, DeWey (1963), Blackman and Gill (1980) and Preston *et al*. (1982) found no relationship between SDS sedimentation value and protein content. Quick and Donnelly (1980) also reported no correlation between SDS sedimentation and protein content in durum wheat.

Several attempts have been made to eliminate the effect of protein concentration on SDS sedimentation (Preston *et al*., 1982; Baik *et al*., 1994; Cubadda *et al*., 2007; Pickney *et al*., 1957). Baik *et al*. (1994) suggested that the SDS sedimentation could be optimized by running the test on a constant protein basis to determine the effect of protein quality, independent of protein content, and to differentiate better among flours.

The differential response must be considered if any correction for protein concentration is to be undertaken. For example, when using the whole meal SDS sedimentation test to differentiate among lines, strong gluten soft wheat lines at 9% protein concentration may have the same SDS sedimentation as a weaker gluten line at 13% protein concentration and it will be impossible to differentiate between them. Lines could be grown in multiple environments to produce grain with different protein concentrations, and SDS sedimentation for all lines could be corrected using the adjusted means from analysis of covariance. However, in early generation testing, the response trend of a particular line would not be known, and multiple location testing is impossible due to limited grain supplies (Carter *et al*., 1999).

Cubadda *et al*. (2007) recommended that the SDS sedimentation test be divided by the protein content and then be multiplied by 10 in order to eliminate the bias caused by the influence of protein content in durum wheat. The corrected SDS sedimentation had an improved correlation with the gluten index and W alveograph value, ranging from 0.86 to 0.93 ($P \leq 0.01$) and from 0.86 to 0.94 ($P \leq 0.01$), respectively. Pickney *et al*. (1957) suggested

that corrections for protein content be made by dividing the sedimentation value by the protein content to give a sedimentation ratio or specific value, but Atkins *et al*. (1965) concluded that the specific value did not show any advantage over the sedimentation value.

In breeding programs, protein content is treated as a separate objective to bread-making quality and therefore the SDS sedimentation test has been criticized for its dependence on protein content (Lorenzo and Kronstand, 1987). Since protein quantity is an important factor in the determining of the quality of flour, it is legitimate to ask whether the SDS sedimentation should not simultaneously measure this factor (Krattiger and Law, 1991).

2.3.6.3 Dough strength

Higher SDS sedimentation is correlated with stronger dough (Dexter *et al*., 1980; Lorenzo and Kronstad, 1987). This was confirmed by Graybosch *et al*. (1996), who reported that increased flour protein content and SDS sedimentation correlate with stronger dough and larger loaf volumes. This makes sense, since the glutenin in gluten determines the SDS sedimentation and it is this proportion of these storage proteins which influences certain dough characteristics like dough development time, maximum dough resistance, dough extensibility, particle size index (Eagles *et al*., 2002) and quality parameters like loaf volume (Payne, 1987).

Zeleny *et al*. (1960), reported a significant correlation (r=0.74) between SDS sedimentation and mixing properties, which was confirmed by Sunderman *et al*. (1965) with correlations of r=0.139 (F2 generation) and r=0.337 (F3 generation) between dough development time and SDS sedimentation and r=0.142 (F2 generation) and r=0.662 (F3 generation) between dough mixing time and SDS sedimentation. Lebsock *et al*. (1964) reported a correlation of r = 0.61 to 0.74 (P≤0.01), while Atkins *et al*. (1965) found a correlation of 0.77 in F7 from 170 F5 derived lines of the wheat hybrid Tascosa x selection 55C1304. In accordance, Fowler and de la Roche (1975a) reported a correlation of r=0.58 (P≤0.01). In durum, Dexter *et al*. (1980) of r=0.69 (P≤0.01), reported a significant correlation between SDS sedimentation and mixograph development in durum wheat; Baik *et al*. (1994) reported correlations of r=0.67 to r=0.85, while Krattiger and Law (1991) reported correlation coefficients between r=0.76 to r=0.69 (P≤0.001) between mixograph peak time and SDS sedimentation depending on the sample size (5g and 1.5g).

Subunits Dx5+ Dy10 have been associated with a high SDS sedimentation and higher dough strength than subunits Dx2+Dy12 at the Glu-D1 locus in most of the genotypes (Saint Pierre *et al*., 2008).

The sedimentation test might be a useful and effective tool to predict dough characteristics in early generations, because of the high positive correlation of sedimentation tests with dough strength parameters (Zeleny, 1947; Zeleny *et al*., 1960; Zeleny 1962; DeWey, 1963; Lebsock *et al*., 1964, Fowler and de la Roche, 1975a; Blackman and Gill, 1980; Quick and Donnelly, 1980). Preston *et al*. (1982) reported that environmental factors appeared to play a less important role (than protein content) in the ability of the SDS sedimentation test to predict dough strength parameters.

The sedimentation test proved to be a useful and simple tool for making early generation wheat selection for desirable dough strength, regardless of how well or poorly the sedimentation test might correlate with other wheat quality characteristics. More than half of the wheat could be eliminated on the basis of sedimentation values without sacrificing a single one of the wheats with long enough mixing tolerance (Zeleny *et al*., 1960). It was concluded that early-generation selection for dough properties should result in substantial genetic advance (Lebsock *et al*., 1964). It might be inadequate to identify a single variable representative of the complex of quality traits, since these traits respond to environmental effects and may be correlated (Van Lill *et al*., 1995a).

Sunderman *et al.* (1965) reported a highly significant negative association of protein content with dough-mixing time and dough type when the effect of sedimentation value is removed. If this is a common situation, an attempt to improve baking quality (loaf volume, grain and texture) by selecting for high-protein content among lines that have the same sedimentation value may actually result in the selection of those lines having weaker mixing properties.

In contrast to this, Quick and Donnelly (1980) found no correlation between SDS sedimentation and mixogram score in durum wheat, while Ayoub *et al*. (1993) found no significant correlation between SDS sedimentation and dough development time for different cultivars.

2.3.6.4 Grain hardness

Carver and Rayburn (1995) speculated that since there is a correlation between SDS sedimentation and grain hardness (thus damaged starch), changes in the biophysical phenomenon (including protein-starch interactions and the extent of the gluten network) might be responsible for environment-dependent changes in SDS sedimentation.

Grain hardness has been extensively studied because of its influence on bread-making quality. Hardness is controlled by a major gene, Ha assigned to chromosome 5D (Groos *et al*., 2004), influencing primarily dough rheological properties (Branlard *et al*., 2001). Glutenin

genes were also found to influence particle size index, which in southern Australia is used as a measure of grain hardness.

The degree of adhesion between starch granules and the endosperm protein matrix probably influences grain hardness. The Ha locus, coding for pin A and pin B genes has been implicated in grain hardness, but other loci are also involved (Sourdille *et al.,* 1996).

It should be kept in mind that common quality relationships within highly selected progeny do not necessarily exist within unselected segregating populations and visa versa (O'Brien and Ronalds, 1984; Fischer *et al*., 1989), therefore O'Brien and Ronalds (1984) stated it is not advisable to make use of indirect quality selection in early generation material on the basis of correlations found within advanced material.

2.3.7 Heritability, dominance and the number of genes involved

The degree of accuracy with which small-scale tests measure genotypic value can be assessed by their heritability (Drucilla *et al*., 1981). For a given characteristic to be effective, a high correlation between the performance of the genotypes selected in early generations and the performance of their progeny in later generations is essential (Baker *et al*., 1977). Heritability from one generation to the next can be calculated by three methods:

-Regression of the F_{x+1} on the Fx plant values

-The standard unit method described by Frey and Horner (1957)

-Use of components of variance obtained from the analysis of variance of F_{x+1} lines

(Sunderman *et al*., 1965)

The heritability of the SDS sedimentation is medium to high (O'Brien and Ronalds, 1987; Kadar and Moldovan; 2003). Lebsock *et al.* (1964) reported a heritability (h^2) of 0.54 in early generations of spring wheat, while Atkins *et al*. (1965) reported h^2=0.54 (F5 on F4 generation), 0.44 (F7 on F4 generation) and 0.63 (F7 on F5 generation) if calculated by doing regression. Sunderman *et al*. (1965) reported that the regression of SDS sedimentation in the F3 on the F2 is 64%. Sunderman *et al*. (1965) reported a significant correlation (0.44) between SDS sedimentation values in the F2 and F3 generation. Matuz (1998) also reported that there is a significant correlation between the SDS sedimentation of the F2 and F3 generations. In contrast to this, Briggs and Shebeski (1971) indicated that regression of the SDS sedimentation values in the F5 was significantly related to the F3 generation for only one in three testing years. Kaul and Sosulski (1964) reported heritability of sedimentation values ranging from 79 to 92%, while O'Brien and Ronalds (1987) reported heritabilities of h^2 =49.6 to 95.8 in the F4 generation of different crosses. Fischer *et al.*

(1989) reported a heritability of 44%. Matuz (1998) reported a broad sense heritability of 0.56 to 0.63 in the F1, F2 and F3 crosses of North American and Hungarian winter wheat varieties, which indicates a rather good heritability. The narrow sense heritability was 0.50 and 0.35 for two different crosses which is regarded as high, since quality is strongly affected by environmental factors, which is taken into account in the calculation, together with the effect of heterosis and epistasis. Kadar and Moldovan (2003) found that the heritability estimates were high for sedimentation index (h^2=0.89) in winter wheat.

In conclusion, SDS sedimentation is influenced by environment, crop year, and their interactions with cultivar (Graybosch *et al*., 1996), but the values are still highly heritable and can be used for selection among early generation progeny (Carter *et al*., 1999; Matuz, 1998). Kaul and Sosulski (1964) studied the inheritance of sedimentation values in the F1, F2, BC1 and BC2 of the spring wheat cross Selkirk X Gabo and reported that SDS sedimentation was partially dominantly determined. In contrast to this, Matuz (1998) reported that dominance of a variety with a lower SDS sedimentation was generally observed in the F1 generation. The means for F2 and F3 cross progeny were similar to their mid-parent values. Heterosis depended on the crop-year and plant density.

Kaul and Sosulski (1964) reported that two genes are responsible for SDS sedimentation volume. In contrast to this, Matuz (1998) reported that one gene was involved in crosses of North American and Hungarian winter wheat varieties. Dhaliwal *et al*. (1987) and Carver and Rayburn (1995) reported that the 1BL/1RS translocation could cause a decrease in SDS sedimentation. The 1B/1R chromosome translocation is widely being used in wheat breeding programs because of the good performance in well-adapted environments and resistance to stripe-, leaf-, and stem rust and powdery mildew that is carried on the short arm of the 1R chromosome segment derived from Petkus rye.

Silvela *et al*. (1993) estimated the contributions of alleles at the homologous high-molecular-weight glutenin subunit (HMW) loci, Glu-A1, Glu-B1 and Glu-D1, to the variation in SDS sedimentation and found less than half of the phenotypic variance to be under genetic control and over a half of this was accounted for by HMW-GS contributions. Initial response to selection was therefore very rapid, as is expected when genes with large effects are involved. In addition, the frequencies of good HMW-GS alleles increased so quickly that their contribution to the genetic variance was exhausted by the fourth generation of selection. The Ax2* allele at locus Glu-A1, as well as Bx7+By8 and Bx7+By9 at locus Glu-B1 and 5+10 at locus Glu-D1, all increased their frequency at the expense of the others. The frequency of allele 5+10 was high in the original population, probably because it is the allele with the greatest effect on quality, and all but one of the original cultivars were selected for good

quality. Possible linkage disequilibria generated by selection and large gene effects or epistasis was, if present, not very important. An unusually large initial response to selection reflects the presence of genes of large effect on the characters. The favorable alleles are rapidly fixed and the variance expressed by them is readily exhausted and response to selection thereafter will be slow.

2.3.8 Experimental design and selection

Assessment of the results of genotype selection trials with appropriate statistical analysis would result in more rapid genetic improvement in the breeding program.

According to Pillay (2000), efficiency of multiple environment screening depends on five factors:

- Germplasm with adequate genetic adaptability potential to the target environments
- Selection sites that are representative of the target environments
- Experimental designs that sample effectively for environmental diversity
- Criteria capable of predicting crop yield potential from evaluation of individual plants in the absence of competition
- Continuation of multiple environment screening after the release of cultivars to exploit newly derived sources of variation arising from the obligatory exposure and adaptation of the genome to the constantly changing environment

Various methods have been proposed to determine the appropriate locations to use in a breeding program to maximize selection response, including reduction of genotype x environment variance by varying combinations of locations (Frey and Horner, 1957) grouping of environments with similar genotype x environment using cluster analysis (Lin and Morrison, 1992) and additive main effects multiplicative interaction (AMMI) analysis (Gauch and Zobel, 1996). Statistical analysis of multi-environment trials will eliminate much of the unexplainable data beneficial in the genotype x environment study.

Analysis of variance

Phenotypic performance of genotypes in multi-environments can be used to calculate the amount of variation attributed to genotypic effects, environmental effects, genotype x environment interaction effects and experimental error (Basford and Cooper, 1998). Variation in SDS sedimentation can be divided in additive main effects for genotypes and environments and non-additive effects due to genotype x environment interaction (reviewed by Pillay, 2000). Genotype mean is a function of genotype by locality (GxL), genotype by year (GxY) and genotype by year by locality (G x Y x L) interaction:

$Y_{ij}=\mu+G_{i+}\ E_{j+}\ (GE)_{ij+}\ \epsilon_{ij}$

μ - general mean

$G_{i,}\ E_{j,\ and}\ (GE)_{ij}$ - the effect of the i^{th} genotype with the j^{th} - environment respectively

ϵ_{ij} - random error

Estimation of the interaction is important to estimate the genotype effects and optimal allocation of resources. The variance components can be calculated by the estimated mean squares from the observed mean squares in the analysis of variance. The higher the component, the more influence it has on the detected variation (Pillay, 2000).

AMMI

Gauch and Zobel (1996) reviewed the AMMI model and reported a dramatic increase in trial efficiency by using this analysis. AMMI analysis should be the first analysis done on multi-environment trials as it includes both multiplicative and additive main effects in the analysis. Pillay (2000) reported that the AMMI is more effective in capturing the sum of squares attributable to the genotype x environment interaction than the linear regression analysis.

Adaptability (average performance across localities) and stability (consistent performance across environments) is both of importance to the breeder. According to Pillay (2000) the variance across environments can be used for traits such as quality. The limitation of stability analysis is the influence of the cultivars, because large deviations from regression do not necessarily imply instability, but it could be that a specific genotype only reacts differently from the rest of the genotypes.

The contribution of additive variance to the performance stability and total variance of a quality character makes the use of fixed criteria or norms for classification purposes impractical. Therefore cultivars are used as biological quality standards and acceptable deviations from the standard are established as classification norms. A standard procedure in South African breeding programs is to do independent culling in later generations with absolute limits of acceptability relative to a check as prescribed by the South African Grain Laboratory (SAGL) (Koekemoer, 2004). In the dry land wheat sector cultivars Betta-DN and Elands are used as quality standards.

Performance stability and experimental design

Performance stability is a complex trait that also exists in early generation material in which multiple genes interact on many levels in reaction to biotic and abiotic stresses. Therefore the screening of early generation material exposed to biotic and abiotic diversity encountered

across production environments is an integral part of any successful breeding program. Replication is essential as it reduces the proportion of variation amongst genotypes due to non-genetic causes (O'Brien, 1983).

Pillay (2000) reviewed previous definitions of performance stability from literature and reported that a genotype is considered stable if the variation over combined environments is small, the deviation mean square from the regression is small and the mean square over combined years is small and the performance across environments is parallel to the mean of all the genotypes. The first two definitions resulted in more effective selection, since they have a genetic and not statistical basis.

The basic criterion for choosing a selection design should be its capacity to sample effectively for genotypic heterogeneity and ensure comparable growing conditions for a large number of entries as well as for plants within entries. This is of great importance in early generations, where limited seed makes replications in a trial impossible (Fasoula and Fasoula, 1995). Briggs and Shebeski (1971) also concluded that frequent controls are essential for efficient selection to reduce the limitations of soil heterogeneity, which are important factors in reducing the effectiveness of selection of single plants for single characteristics.

An experimental design making use of a single plant moving mean check has certain advantages because of the minimization of soil heterogeneity and frequent distribution of control plots. Only modest results from the application of moving mean selection are expected when using single plants as checks. The reason for this is twofold. Firstly, the performance of individual plants depends strongly on individual conditions. This starts before sowing. The size of the kernel has an important impact on the ultimate performance of the plant growing from it. At sowing, the kernels are positioned at different depths as well as at different orientations. Next, the seedlings are submitted to different degrees of hindrance by clods, pebbles, manure, remnants of the former crop etc. They are damaged at different levels by mice, rabbits, hares, etc. Their soil-moisture and fertilizer-supply conditions will differ. Especially the conditions in the very beginning of plant growth have lasting effects. Few of the mentioned facts can be controlled (Bos, 1981). As far as such control is not effective, superior phenotypes instead of superior genotypes will be favoured by selection. Thus the results can be disappointing because the moving mean method only eliminates masking environmental effects as far as these are observed over areas larger than those covered by the central plant plus its check neighbours. The second reason for limited results

is the variation in performance that may exist in the check cultivar itself, which may lead to biased data.

Alternation between different locations when conducting early generation allows for the selection of varieties with wider adaptability than selecting in a single environment (Saint Pierre *et al*., 1967).

Another measure of the usefulness of selection among plants for a certain character is to determine what portion of the good F3 lines would have been lost and what portion would have been saved through selection (Sunderman *et al*., 1965). Atkins *et al*. (1965) found that a selection intensity of 13.8% would eliminate testing of inferior quality pure-lines in advanced generations, but such a selection on quality alone would necessitate a large F2 population to afford ample opportunity for selection for other agronomic characters among F2 derived families in later generations. Matuz (1998) showed that SDS sedimentation fulfils this requirement, as selection of 34 F2 plants with high SDS sedimentation values would have resulted in retention of 90% and 71% of the F3 highest sedimentation and protein lines, respectively.

Selection strategy is also of great importance and should be carefully considered. For example, Pearson *et al*. (1981) reported different heritability for milling extraction and Pelshenke wheat meal fermentation time when F4 rows rather than F3 single plants were selected; suggesting single plants might be superior. Although both protein content and SDS sedimentation can be performed on a single plant, little information is available on the improvement of these two quality characteristics that can be made by selection in early generations (Sunderman *et al*., 1965).

Optimum number of locations

Costs and time-consumption can be reduced if the breeder can discard additional localities after determining the number of locations essential for effective selection. The optimum number of locations, years and replications required for effective evaluation and selection can be determined by manipulating the number of locations, years and replications to minimize the variance of the genotype means, which is composed of the components of variance, GxL, GxY and GxLxY interactions and error (Crossa, 1991).

Where the GxE for a trait is large, testing must be done using fewer replicates and more locations and years, even though this results in higher costs (Sprague and Federer, 1951).

The theoretical variance of the mean gives an indication of the number of locations to do effective evaluation.

$$V = \frac{\delta^2_e}{rpy} + \frac{\delta^2_{vpy}}{py} + \frac{\delta^2_{vp}}{p} + \frac{\delta^2_{vy}}{p}$$

With:

r - replications

p - localities

y - years

The smaller the variance of the mean with different combinations of the replications, locations and years, the more accurate the estimate of the genotype performance. By determining the point where the number of locations will result in a minor increase in the estimate of performance accuracy, the optimal amount of localities can be determined (Pillay, 2000).

2.3.9 Expression

In order to express the results of the sedimentation test in terms that tend to reflect only gluten quality, sedimentation value to give an indication of specific volume was expressed in different ways (Zeleny, 1947; Greenaway *et al*., 1966; Kovacs, 1985). Zeleny (1947) expressed the specific volume as and indication of "specific sedimentation", which is the SDS sedimentation divided by the percentage of protein (on a 14% moisture basis):

Vs=(V-K)/P

Vs= Specific volume

V = actual loaf volume (ml)

K = theoretical volume of a loaf made from flour containing no protein

P = percent of protein in the flour (14% moisture basis)

The value of K was determined by extrapolating the regression line of loaf volume against protein percentage to a protein value of zero percent. The value obtained was 160ml and close to the actual loaf volume obtained by baking a loaf of bread in which starch was substituted for the flour in the bread formula. Specific sedimentation (SDS sedimentation divided by protein percentage) is a useful measure of gluten quality (Zeleny, 1947).

Greenaway *et al*. (1966) calculated the sedimentation values to a 12% protein basis by using the following regression equation from Zeleny (1962) based on the analysis of large numbers of wheat samples:

SV = 5.83 (%PC - 1.0) - 24.5

SV = sedimentation value

PC = protein content

This regression equation showed that wheat containing 12% protein on a 14% moisture basis will on average have a sedimentation value of 40 and that at higher or lower protein levels the sedimentation value will on average be 5.83 units higher or lower that 40 for each 1.0% protein. The reason for using the sedimentation value is to minimize or eliminate the differences in wheat protein content that can be ascribed to specific genotype.

Kovacs (1985) used a sedimentation ratio (Sr), to eliminate the tendency of different cultivars to perform differently at different locations, where

Sr = St/Ss

St = height of sediment of test sample

Ss = height of sediment of standard

With this calculation, the SDS sedimentation is expressed as a ratio of the standard and eliminates the need to correct for small fluctuations in temperature, particle size (due to different mills) and other laboratory conditions both within and among laboratories, which may affect the sedimentation value.

2.4 Summary

SDS sedimentation appears to have considerable value in selection for quality in a pedigree or modified bulk breeding program (Atkins *et al*., 1965; Kaul and Sosulski, 1964; Lebsock *et al*., 1964; Zeleny *et al*., 1960). The SDS sedimentation and protein content tests can be performed on grain from a single plant (Sunderman *et al*., 1965) with still enough seed remaining to plant.

According to Greenaway *et al*. (1966), and Fowler and de la Roche, (1975c) SDS sedimentation is the test giving the best singular prediction of baking potential and strength for hard wheat. Although protein content is known to influence SDS sedimentation, the extent of the influence is variety-dependent and reflective of the protein quality. It could be considered as beneficial that the sedimentation test “captures” both sources of variation: protein quality and quantity, where protein content could be an additional factor upon which

to interpret a given specific sedimentation volume (Morris *et al.*, 2007). The test could be used with ease on early generation material, as early as F2 and F3 generation plants, while simultaneously providing opportunities for observations on disease reaction and agronomic characteristics (Atkins *et al.*, 1965).

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CHAPTER 3

RELATIONSHIP OF THE SDS SEDIMENTATION TEST WITH BAKING VOLUME

3.1 INTRODUCTION

In early generations, only a small amount of wheat is available and information related to the quality of the wheat is needed immediately, making the production of baking products to assess baking volume impractical. Therefore several predictive tests that are closely related to wheat quality are used to assess quality attributes (Colombo et al., 2008). Since high loaf volume is one of the most important quality aims in South African wheat breeding programs, a trait that serves as a good indication of bread volume would be relevant and useful as selection criteria in early generations.

Many international studies have indicated a high correlation between SDS sedimentation and loaf volume (Zeleny, 1947; Pickney et al., 1957; Atkins et al., 1965; Sunderman et al., 1965 Orth et al., 1972; Fowler and de la Roche, 1975, Axford et al.; 1978; McDermott and Redman, 1977; Blackman and Gill, 1980; Dexter et al., 1980; Moonen et al., 1982; Preston et al., 1982; Lorenzo and Kronstad, 1987; Krattiger and Law, 1991; De Villiers and Laubscher, 1995; Grausgrauber et al., 2000; Barnard et al., 2002; Colombo et al., 2008) and SDS sedimentation is therefore regarded as a reliable predictor of loaf volume.

The SDS sedimentation test measures the sedimentation volume of a suspension of flour in diluted lactic acid, which is related to the swelling of glutenins where glutenin is associated with the bread making quality of flours (Eckert et al., 1993). However, SDS sedimentation should not be interpreted as a measurement of loaf volume unless the corresponding protein content values are taken into consideration (Lorenzo and Kronstad, 1987; Cubadda et al., 2007), because protein content influences the correlation between SDS sedimentation and loaf volume (Preston et al., 1982; Carter et al., 1999). Samples with lower protein content (below 13%) usually exhibit the strongest correlation between SDS sedimentation and loaf volume (Fowler and de la Roche, 1975). Samples with protein content over 14%, however showed no significant correlation between SDS sedimentation and loaf volume (Preston et al., 1982).

Ayoub et al. (1993) reported a correlation coefficient between $r=-0.21$ and 0.64 ($P \leq 0.01$) between SDS sedimentation and bread loaf volume (cm^3) for different cultivars, therefore correlations differ between cultivars. In three of the cultivars, correlations improved when calculated independently from the protein content, confirming that the latter has an influence

on the sedimentation value. Sapirstein and Suchy (1999) reported a correlation of r=0.97 and 0.94 between SDS sedimentation and loaf volume for ground wheat and sieved ground wheat respectively, so the type of flour used may also influence results. It should be kept in mind that common relationships within advanced material (highly selected progeny) do not necessarily exist within unselected segregating populations (Fischer et al., 1989).

The purpose of this study was to determine the relationship between baking volume and SDS sedimentation value and other quality characteristics in a set of hard red bread wheat genotypes in different locations. In the following chapters there was not enough material to do baking tests, so the trials in this chapter were done to determine whether findings in other studies on the strong relationship between baking volume and SDS sedimentation volume is also true for winter wheat under South African dryland production conditions in the Free State.

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3.2 MATERIAL AND METHODS

3.2.1 Cultivation

Three intermediate hard red wheat elite lines, in their final phase of evaluation in the breeding program, were evaluated over four locations in the Eastern Free State together with Elands and Gariep, the two cultivars used as standard checks. The lines and their pedigrees are presented in Table 3.1. Planting dates are presented in Table 3.2. The trial was planted in a complete randomized block design with four replications. A modified Gaspardo precision planter was used for planting. Plots consisted of five rows of 5m in length with a spacing of 50cm between rows. This resulted in a plot size of 6.75m^2. The spacing within rows was ±30cm. All the seed was treated with Vitavax Plus and Gaucho prior to planting. A fertilizer application of a 3:2:1(25) mixture was used for all trials. Fertiliser was applied at a rate of 35 kg Nha^{-1}. Russian wheat aphid and weeds were controlled on all trials with a tank mixture of Harmony M and Metasystox-R. The middle three rows of each experimental plot were harvested with a Wintersteiger plot harvester when full harvest maturity was reached. Samples were dried to a moisture content of about 12% before samples were individually cleaned prior to grain yield and quality determination.

Table 3.1 Two intermediate cultivars and nine lines used in this study

Genotype	ID	Pedigree
1	Elands (L1)	Elands
2	Gariep (L2)	Gariep
3	L3	SST333//661L 1-33/Tugela-DN
4	L4	Gar*2//Kar/P3401-6-4
5	L5	Gariep/4/SA463/*4 Gamtoos/3/YD"S"/Bon//Dove

Table 3.2 Planting dates in 2007

Location	Planting date	Latitude	Longitude
Meets	18/2007	28° 06'42''S	28° 07'23''E
Clarens	19/6/2007	28° 23'53''S	28° 25'48''E
Bethlehem 1	18/6/2007	28° 09'14''S	28° 17'17''E
Kransfontein	19/6/2007	28° 06'45"S	28° 37'41"E

3.2.2 Parameters measured

3.2.2.1 Baking volume

The approved AACC 10-11 method was followed (AACC, 2000) which is the sponge-dough or pound loaf method. Results were reported as baking loaf volume (cm^3).

3.2.2.2 SDS sedimentation

The approved AACC 56-70 method was followed (AACC, 2000), with certain modifications. Sample weight and SDS concentration were standardized. A whole meal sample of 5g was used. The sample was ground with a Perten 3100 laboratory mill, containing a standard sieve of 0.8mm. The SDS volume technique was performed within 5 hours of grinding, at a stable room temperature of 24℃. All the chemical solutions were made up the day before the experiments were conducted. The SDS value was recorded after 30 minutes. Results were reported as sedimentation volume (ml/5g).

3.2.2.3 Protein content

Grain protein content was determined by near-infrared reflectance spectroscopy (Infra Alyser, 360, Bran & Luebbe, Hamburg; AACC, 2000). Flour protein content was determined according to the AACC 39-11 method (AACC, 2000).

3.2.2.4 Mixing development time

Mixing development time was determined according to AACC 54-40A (AACC, 2000) with a 35g National Mixograph (National Mfg. Lincon, Nebraska). The mixing development time over a period of 6 minutes was recorded.

3.2.2.5 Grain yield

Harvesting of the three middle rows of each plot was done and the mass was converted from g m^{-2} to ton ha^{-1} to determine grain yield.

3.2.2.6 Flour yield

All the wheat samples were milled in a laboratory pneumatic mill, Bühler model MLU-202 (manufactured by Bühler Bros., Inc., Uzwil, Switzerland). The AACC 26-21A method for milling hard wheat was followed (AACC, 2000).

3.2.3 Statistical analysis

3.2.3.1 Analysis of variance

The combined analysis of variance was conducted to test the significance of genotype, environment and their interactions across locations. The ANOVA was carried out for baking volume and SDS sedimentation. Agrobase (2005) was utilized to perform the analysis of variance

3.2.3.2 Correlation

The relationship between the parameters was assessed by Pearson's test using Agrobase (2005).

3.3 RESULTS AND DISCUSSION

3.3.1 Analysis of variance

3.3.1.1 Baking volume

The influence of genotype was highly significant when analysing the combined trials (Table 3.3). The significant genotype effect suggests consistent differences in performance amongst the genotypes. Locations also had a highly significant influence on the performance of the genotypes as can be seen by the significant location and genotype x location mean squares, indicating that there is an interaction between the environment and genotypes.

Table 3.4 ranked the mean genotype performance of the genotypes at three locations. The loaf volume of L1 was significantly higher than that of the rest of the genotypes, except for L3. The loaf volume of L5 was significantly lower than that of the rest of the genotypes.

3.3.1.2 SDS sedimentation

The influence of genotype and location was highly significant when analyzing the combined trials (Table 3.3). The significant genotype effect indicates large differences in performance amongst the genotypes. Locations also had a highly significant influence on the performance of the genotypes as can be seen by the significant location and genotype x location interaction, indicating that there is an interaction between the environment and genotypes. It can therefore be concluded that testing of the genotypes needs to be conducted at different locations for a reliable evaluation of genotype performance.

Eisemann *et al*. (1990) stated that it is the part of GxE interaction which leads to rank changes which impedes response to selection as it can change the composition of the

selected and rejected groups. Table 3.5 ranked the mean genotype performance of the genotypes at three locations. The SDS sedimentation of L1 was significantly higher than that of the rest of the genotypes. This genotype also ranked highest in loaf volume. The same genotype that ranked second in loaf volume (L3), ranked second in SDS sedimentation. The ranking of the lowest three SDS sedimentation genotypes differed from the loaf volume ranking. It can be concluded that there were small differences in ranking between SDS sedimentation and loaf volume, but that the genotypes that ranked high in SDS sedimentation also ranked high in loaf volume. The genotypes that ranked low in SDS sedimentation also ranked low in loaf volume. Therefore little difference would have resulted in the composition of selected and rejected groups by using SDS sedimentation rather than loaf volume. The high repeatability value (b-value) of SDS sedimentation is very encouraging, since this is a major requirement of a selection test.

Table 3.3 Combined analysis of variance

		Baking volume (cm^3)		
Source	**df**	**Sum of squares**	**Mean squares**	**Significance**
Total	79	455720.00		
Replications in locality	12	11967.50	997.292	
Genotype	4	100426.25	25106.56	**
Location	3	289562.50	96520.83	**
Genotype x location	12	29306.25	2442.19	**
Residual	48	24457.50	509.53	
Grand mean = 766.00			CV (%) = 2.95	
R-squared = 0.95			b value = 0.90	
** P ≤ 0.01				

		SDS sedimentation (ml/5g)		
Source	**df**	**Sum of squares**	**Mean squares**	**Significance**
Total	79	13231.99		
Replication	12	137.350	11.47	
Genotype	4	11266.68	2816.67	**
Location	3	938.74	312.91	**
Genotype x location	12	361.33	30.11	*
Residual	48	527.60	10.99	
Grand mean = 57.51			CV (%) = 5.76	
R-squared = 0.96			b value = 0.90	

** P ≤ 0.01, * P ≤ 0.05

Table 3.4 Baking volume (cm^3) of the genotypes in 2007

Genotype	Baking volume (cm^3)	Rank
L1	804.06a	1
L2	779.69bc	3
L3	792.50ab	2
L4	747.81c	4
L5	705.94d	5

LSD= 13.39 Means followed by different letters are significantly different at P ≤ 0.05

Table 3.5 SDS sedimentation (ml/5g) of the genotypes in 2007

Genotype	SDS volume (ml/5g)	Rank
L1	68.56a	1
L2	53.06d	4
L3	66.00b	2
L4	36.25e	5
L5	63.69c	3

LSD= 1.97 Means followed by different letters are significantly different at P ≤ 0.05

3.3.2 Correlation

As the environment affects the protein content of the grain, it may also affect the SDS sedimentation (Lorenzo and Kronstad, 1987) and bread volume, since the protein content of the grain determines these parameters. Different correlation coefficients between the same parameters can therefore be expected at different locations (De Villiers and Laubscher, 1995).

There was a highly significant correlation between loaf volume and SDS sedimentation ($r=0.53$, $P\leq0.01$) (Table 3.6), which is in accordance with reports by various authors (Orth *et al*., 1972; Moonen *et al*., 1982; Lorenzo and Kronstad, 1987; Krattiger and Law, 1991; De Villiers and Laubscher, 1995; Khatkar *et al*., 1996; Colombo *et al*., 2008). The correlation was lower than that reported by Orth *et al*. (1972) ($r=0.88$, $p\leq0.01$).

There was a highly significant correlation between loaf volume and protein content ($r=0.70$), which is in accordance with other reports (Busch *et al*., 1969; Tipples and Kilborn, 1974; Bhatt and Derera 1975; De Villiers and Laubscher, 1995; Colombo *et al*., 2008 and Wang and Kovacs, 2002).

There was no significant correlation between loaf volume and mixing development time, which is in contrast with the report of Busch *et al*. (1969). A possible explanation for the absence of any association between loaf volume and mixing development time is that this association is genotype dependent and varies according to the protein quantity and gluten composition.

The association between SDS sedimentation and protein content was not significant although it is well known from literature that the protein content influences SDS sedimentation (Orth *et al*. 1972; Moonen *et al*., 1982; Dick and Quick, 1983; Lorenzo and Kronstad, 1987; de Villiers and Laubscher, 1995; Finney and Bains, 1999; Carter *et al*., 1999; Cubadda *et al*., 2007; Morris *et al*., 2007; Colombo *et al*., 2008). A possible explanation for the lack of association in this study between SDS sedimentation and protein content may be that the influence of protein content is genotype dependent and reflective of the protein quality and therefore varies.

The relationship between SDS sedimentation and mixing development time ($r=0.34$, $P\leq0.01$) was highly significant. Selection of higher SDS sedimentation volumes may result in stronger dough characteristics in the following generations of certain genotypes, which is not always preferable in all breeding programs, since dough of a medium strength is preferred in the South African market.

Table 3.6 Phenotypic correlation coefficients including all the genotypes in 2007 from four locations in the Eastern Free State

Trait	LV	SDS	FPC	MDT	YLD	FLY
Loaf volume (LV)	1.00					
SDS sedimentation (SDS)	0.53 **	1.00				
Flour protein content (PC)	0.70 **	0.17	1.00			
Mixing development time (MDT)	-0.16	0.34 **	-0.49 **	1.00		
Grain yield (YLD)	0.08	-0.00	0.00	0.03	1.00	
Flour yield (FLY)	0.36 **	0.21	0.26 *	0.05	0.39 **	1.00

**** P≤0.01, * P≤0.05**

3.4 CONCLUSIONS

There was a highly significant correlation between loaf volume and SDS sedimentation ($r=0.53$, $P \leq 0.01$), which is in accordance with findings in studies in other studies. (Orth *et al*., 1972; Moonen *et al*., 1982; Lorenzo and Kronstad, 1987; Krattiger and Law, 1991; De Villiers and Laubscher, 1995; Khatkar *et al*., 1996; Colombo *et al*., 2008).

Both genotype and location and their interaction significantly influenced baking volume and SDS sedimentation. The individual rankings of SDS sedimentation and baking volume differed, but the two genotypes ranking the highest in one trait, also ranked highest in the other. The three genotypes ranking lowest in loaf volume also ranked lowest in SDS sedimentation volume. However, if values above 60ml were accepted, the SDS sedimentation test may have eliminated a line with intermediate, acceptable loaf volume (L2), and may have selected the line with poorest LV (L5). The test therefore have value in early generations where the SDS sedimentation is wide, to enable the breeder to outselect the poorest lines. Its value as a singular test if the variation is small is, however, limited. If looking at the relationship of the different traits, SDS sedimentation had a highly significant relationship with baking volume and would serve as a good indication of this trait for selection.

Conclusions reached are limited to the population and environment studied. The population size used was smaller than originally envisaged and this could allow random influences to bias some relationships. The materials used were already subjected to several years of selection and this could also result in biased relationships.

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CHAPTER 4

GENOTYPE X ENVIRONMENT INTERACTION FOR THE SDS SEDIMENTATION TEST AND ITS ABILITY TO DISCRIMINATE BETWEEN HARD RED WINTER WHEAT GENOTYPES

4.1 INTRODUCTION

The Free State winter wheat region is the largest contributor to the national wheat crop of South Africa. The best wheat for bread making is hard red winter wheat that possess relatively long dough mixing time, high flour extraction (milling yield), good mixing tolerance (dough handling), high loaf volume and superior internal bread making properties (Van Lill *et al*., 1995). Priority is given to the quality aspects of the wheat in breeding programs in South Africa because of the strict regulations for cultivar release. Currently high numbers of genotypes are unnecessarily being carried from one to the next generation in the early generations, only to be discarded in the advanced generations because of falling short of the strict quality standards. If a more simple and reliable test could be used in the early generations of the program, a more effective way to do culling of quality poor lines would be possible.

There are a few factors, however, which complicate the utilization of a single quality trait in early generation selection. It is well known that wheat quality depends on the genetic potential of a line, the environmental influence during cultivation and the interaction of genotype and environment (Graybosch *et al*., 1996). It is therefore important to determine and quantify the extent to which a factor like the environment and genotype x environment interaction contributes to variation in each wheat quality parameter. Genotype x environment interaction complicates analysis by confounding the genotypes' observed performance with its true value. Therefore to focus on these parameters alone would hinder effective selection, as the environmental influence would "mask" the true genetic potential of the line, thus decreasing genetic advance. The influence of genotype x environment interaction on a trait makes the assessment of the performance of genotypes through multi-environment trials over years essential, since a generally large component of genotype x environment can be attributed to interactions with years and locations. The extent of the environmental influence on certain quality parameters vary but is generally large for protein content and protein related parameters (Fowler and de la Roche, 1975b). The cause of the interaction is the contribution of the different genes that control the traits that varies in different environments (Pillay, 2000).

Increased knowledge about the effects of the environment on quality parameters should help to determine to what extent a specific quality parameter could discriminate between closely related lines in a breeding program and an improved ability to predict optimum site and year for selection of the specific parameter. In order to minimize the masking effect of genotype x environment interaction and optimize selection, a breeder should determine and select a quality parameter or parameters that perform consistently over environments, therefore has a low variability for the trait over environments. Stability, however, also varies from genotype to genotype. The adaptability of each genotype should also be taken in consideration. If the value of a trait under investigation is high for a genotype relative to the other genotypes, it is considered to be well adapted to the region (Lin and Binns, 1994).

Widely adapted genotypes would have a high average value for the trait under investigation (performance), but low genotype x environment contributions, whereas specifically adapted genotypes would have high average trait values and high genotype x environment interactions or low average trait values and high genotype x environment interactions. Best performing genotypes at optimum locations usually perform poorly in conditions associated with stress and visa versa. By focusing on broad adaptation, genotypes expressing their genetic trait potential will be selected and by focusing on specific adaptation, genotypes with high stability for the trait will be favoured, rather than their potential for the specific trait (Pillay, 2000).

Statistical analysis of multi-environment trials will eliminate much of the unexplainable data beneficial in the genotype x environment study. Significant genotype by location interaction suggests that the locations are very different within the region and that the breeding aim should be to produce a number of varieties for the region, but each exclusively adapted to one of the environments. If genotype by year interaction is significant, years contribute significantly to the unpredictable component of the environment and therefore testing the genotypes over years is necessary. Genotype x year and genotype x year x location interaction is usually larger than genotype x location interaction (Pillay, 2000).

The localities used should represent the target environment of investigation, which is sampled by testing the genotypes at a number of locations over a number of years. The number of locations should be adequate to result in accurate estimates of the genetic merit of the tested genotypes (Pillay, 2000).

Other factors to take into consideration when selecting quality tests to apply in early and later generations are the heritability of the character and correlations with other quality parameters so that selection would not result in a negative influence on other quality parameters. In the

case of two negatively correlated traits of importance, direct selection should be applied, whereas indirect selection can be used if positive correlations exist between the two characters. Correlations may vary according to the environment, season and genotype. For instance, it was reported that the correlation between SDS sedimentation and protein content varies according to the location (De Villiers and Laubscher, 1995), season (Bushuk *et al*., 1969) and genotype (Silvela *et al.,* 1993).

It should also be kept in mind that common quality relationships within highly selected progeny do not necessarily exist within unselected segregating populations and visa versa (O'Brien and Ronalds, 1984; Fischer *et al*., 1989).

The discrimination ability of SDS sedimentation has been evaluated in previous studies, but the material involved has usually been from diverse backgrounds, which broadens the genetic variability and may lead to an increase in discrimination ability in the material. The discrimination ability between closely related lines, as is sometimes the case in a commercial breeding program, has therefore not been adequately investigated. It could be reasoned that a breeding program should always have material with a wide genetic range, but closely related lines often occur in a commercial program when a set of specific crosses has been made with the aim of improving quality or any other specific character. Although the genetic diversity in the program as a whole would be diverse, this would not necessarily be the case in the progeny of this set of parents, because some donor parents would be used more than once on the best adapted genotypes in the program which needs quality improvement. This situation has motivated this study.

The SDS sedimentation test was considered, because in comparison with other quality tests, it is a relatively low-cost, less time-consuming test that requires low manpower and is relatively inexpensive and requires no elaborate laboratory equipment. It has proved to be a reliable, highly reproducible quality test that generally gives a good indication of the end-use quality of wheat (Blackman and Gill, 1980; Carter *et al*., 1999) in cases where the wheat has a low to medium protein content (Krattiger and Law, 1991). There has been evidence that SDS sedimentation is the test that singularly gives the best prediction of bread baking potential and strength for hard wheat (Greenaway *et al*., 1966).

SDS sedimentation has also been chosen, because it accurately assesses protein quality (Carter *et al*., 1999) and quantity (Zeleny, 1947) and has successfully been applied in breeding programs before (Blackman and Gill, 1980; Carter *et al*., 1999; Dick and Quick, 1983). It has been criticized for its dependence on protein quantity, however (Preston *et al*., 1982; Lorenzo and Kronstad, 1987). Although it would be advantageous to break this

relationship for research purposes, the fact that SDS sedimentation gives a good indication of the general protein quality and quantity qualifies it as an effective selection tool in any breeding program. Because of its dependency on protein content, SDS sedimentation should not be interpreted as a measurement of loaf volume unless the corresponding protein values are taken into consideration (Lorenzo and Kronstad, 1987). Therefore, an extra SDS sedimentation value (SV), which is the SDS sedimentation divided by the protein content, was also included in this investigation.

The aim of this study was:

1. To establish the significance of genotype x environment interaction on SDS sedimentation in the advanced generations of hard red winter wheat
2. To determine the discrimination ability of SDS sedimentation between closely related genotypes in advanced generations
3. To compare SDS sedimentation and SDS value (SV)
4. To study the relation of SDS sedimentation with other quality traits in advanced generations
5. To determine which traits contribute the most to the variation in SDS sedimentation by performing multiple stepwise regression
6. To determine the optimal number of locations and years to effectively select for SDS sedimentation

4.2 MATERIAL AND METHODS

4.2.1 Cultivation

Nine hard red wheat elite lines and two hard red cultivars were evaluated for three consecutive years over eight locations in the Eastern Free State, a dry land wheat production area where wheat is planted as an alternative winter crop to maize, which is cultivated in summer. The lines involved were in their final phase of evaluation in the breeding program (Table 4.1). Four lines were included in the elite phase in 2003 and five in 2004. Four of these lines were sister lines with identical pedigrees. Elands and Komati were the two cultivars used as standard checks. Although it would be ideal to select unrelated genotypes for discrimination purposes in a study, the lines were selected to represent the status of a true commercial program and to determine the ability of SDS sedimentation to effectively discriminate between closely related lines, as is sometimes the case in a commercial breeding program.

Planting dates are presented in Table 4.2. A modified Gaspardo precision planter was used. Plots consisted of five rows of 5m in length with a spacing of 50cm between rows. This

resulted in a plot size of 6.75m^2. The spacing within rows was ±3cm. All the seed was treated with Vitavax Plus and Gaucho prior to planting. A fertilizer application of a 3:2:1(25) mixture was used for all trials. Fertilizer was applied at a rate of 35 kg Nha^{-1}. Russian wheat aphid and weeds were controlled on all trials with a tank mixture of Harmony M and Metasystox-R. The middle three rows of each experimental plot were harvested with a Wintersteiger plot harvester when full harvest maturity was reached. Samples were dried to a moisture content of about 12% before samples were individually cleaned prior to grain yield and quality determination.

Table 4.1 Two cultivars and nine lines used in this study

Genotype	ID	Pedigree
1	Elands	Elands
2	Komati	Komati
3	G1	Saulesku3//SA1684/*5 Betta (11)
4	G2	TJB 368-251/*2BULS//Gariep
5	G3	Lov29/3/FTG/SPWX//AFGHH996/MX120/4/Tugela-DN
6	G4	SST333//661L 1-33/Tugela-DN
7	G5	Gariep*2//Karee/P3401-6-4
8	G6	Gariep*2//Karee/P3401-6-4
9	G7	Gariep*2//Karee/P3401-6-4
10	G8	Gariep*2//Karee/P3401-6-4
11	G9	Gariep/3/Kavkaz/Jaral"S"//Flicker"S"

Table 4.2 Planting dates from 2004 to 2006

	Year		
Location	**2004**	**2005**	**2006**
Clocolan	17/6/2004	22/7/2005	25/7/2006
Clarens	23/7/2004	22/7/2005	26/7/2006
Bethlehem 1	26/7/2004	25/7/2005	27/7/2006
Bethlehem 2	26/7/2004	25/7/2005	27/7/2006
Meets	20/7/2004	20/7/2005	21/7/2006
Petrus Steyn	20/7/2004	21/7/2005	21/7/2006
Reitz	21/7/2004	21/7/2005	21/7/2006
Kransfontein	23/7/2004	21/7/2005	24/7/2006

4.2.2 Parameters measured

4.2.2.1 Protein content

Grain protein content was determined by near-infrared reflectance spectroscopy (Infra Alyser, 360, Bran & Luebbe, Hamburg; AACC,2000). Flour protein content was determined according to the AACC 39-11 method (AACC, 2000).

4.2.2.2 Mixing development time

Mixing development time was determined according to AACC*54-40A (AACC, 2000) with a 35g National Mixograph (National Mfg. Lincoln, Nebraska). The mixing development time over a period of 6 minutes was recorded.

4.2.2.3 Falling number

Grain samples of 50g were milled with a Falling number KT-120 laboratory mill (0.8mm) sieve. Then Hagberg Falling number was determined according to the procedures of AACC*56-81B (AACC, 2000). No corrections for moisture content were made, since the sample weight was calculated according to its moisture content.

4.2.2.4 SDS sedimentation

The approved AACC*56-70 method was followed (AACC, 1983), with certain modifications. Sample weight and SDS concentration were standardized. A whole meal sample of 5g was used. The sample was ground with a Perten 3100 laboratory mill, containing a standard sieve of 0.8mm. The SDS sedimentation volume technique was performed within 5 hours of grinding, at a stable room temperature of 24°C. All the chemical solutions were made up the day before the experiments were conducted. The SDS sedimentation was recorded after 30 minutes. Results are reported as sedimentation volume (ml/5g).

4.2.2.5 SDS value (SV)

SV was calculated according to the method described by Zeleny (1947) expressing the SDS sedimentation divided by the flour protein content (on a 14% moisture basis) on a percentage basis.

4.2.2.6 Grain yield

Harvesting of the three middle rows of each plot was done and the mass was converted from g m^{-2} to ton ha^{-1} to determine grain yield.

4.2.3 Statistical analysis

4.2.3.1 Analysis of variance

The combined analysis of variance was conducted to test the significance of genotype, environment, year and their interactions across years and locations. The ANOVA was carried out for SDS sedimentation, SV, protein content, mixing development time, flour yield and grain yield. It was carried out on the individual locations (years combined) to compare genotype means. Cultivar differences were determined by means of the least significant difference (LSD, $P \leq 0.05$). Agrobase (2005) was utilized to perform the analysis of variance, from which correlations were derived.

Table 4.3 Expected ANOVA and expected mean squares (from Rasmusson and Lambert, 1961)

Source	d.f.	Mean square	Expected mean square
Replication	ly(r-1)		
Genotype	(g-1)	M_5	$\delta^2_e+r\delta^2_{gly}+ry\delta^2_{gl}+rl\delta^2_{gy}+rly\delta^2_g$
Location	(l-1)		
Year	(y-1)		
Genotype x year	(g-1)(y-1)	M_3	$\delta^2_e+r\delta^2_{gly}+rl\delta^2_{gy}$
Genotype x location	(g-1)(l-1)	M_4	$\delta^2_e+r\delta^2_{gly}+ry\delta^2_{gl}$
Genotype x year x location	(g-1)(y-1)(l-1)	M_2	$\delta^2_e+r\delta^2_{gly}$
Error	ly(g-1)(r-1)	M_1	δ^2_e

M - mean square, Y - year, L - location, R - replication, G - genotype, GxY - genotype x year interaction, GxL - genotype by location interaction, GxLxY - genotype by year by location interaction

Table 4.4 Calculations to determine variance components (Rasmusson and Lambert, 1961)

Variance component	Calculation
Genotype (δ^2_g)	$\frac{M_5 + M_2 - M_3 - M_4}{r\ l\ y}$
Genotype x location (δ^2_{gl})	$\frac{M_4 - M_2}{r\ y}$
Genotype x year (δ^2_{gy})	$\frac{M_3 - M_2}{r\ l}$
Genotype x location x year (δ^2_{gly})	$\frac{M_2 - M_1}{r}$
Plot error (δ^2_e)	M_1
Phenotype (δ^2_p)	$\delta^2_g +(\delta^2_{gl}/l)+(\delta^2_{gy}/y)+(\delta^2_{gly}/ly)+(\delta^2_e/rly)$
Heritability (b)	$\frac{\delta^2_g}{\delta^2_p}$

M - mean square, δ^2_p - phenotypic variance, δ^2_g - genotypic variance, δ^2_{gl} - genotypic x location interaction, δ^2_{gy} - genotypic - x year interaction, δ^2_{gly} - genotypic x location x year interaction, δ^2_e - environmental variance, y - number of years, l - number of locations, r - number of replications

4.2.3.2 Canonical variate analysis

The canonical variate analysis (CVA) is an univariate analysis of variance technique whereby the statistical evidence for differences between cultivars or environments can be shown for each variate or trait separately, but does not give an indication of how the genotypes group or which variates are most important in discriminating between groups. Canonical variate analysis is used when it is of interest to show differences between groups rather than between individuals (Van Lill *et al*., 1995; De Lange and Labuschagne, 2000).

The canonical approach allows the explanation of relationships in a data set between two large sets of variables. The variables are reduced to a smaller set of variates that account for most of the variability in the data set. These new sets of variables are linear combinations of the original measurements, and are thus indicated as vectors of loading for the original measurements (Graybosch *et al*., 1996). A set of directions is obtained in such a way that the ratio of between-group variability to within-group variability in each direction is maximized (Van Lill *et al*., 1995). The horizontal separation (on the x-axis) represented the one set of canonical variates (CV1) and the vertical separation (on the y-axis) represented the other set of canonical variates (CV2). The canonical correlation expresses the overall degree of relationship between the two groups of constructed variates.

Canonical variate analysis was used to determine which characteristics discriminate most between the cultivars. In this study, the variates represent the quality and yield characteristics that were measured on wheat genotypes over 11 different environments in the Eastern Free State for three consecutive years. The data were analysed, using GenStat (2000). The radius of the circles represents the square root of the 95% point of a chi-squared variable. Since the plot is two dimensional, the degree of freedom equals two.

4.2.3.3 Additive Main effects and Multiplicative Interaction

Additive Main effects and Multiplicative Interaction (AMMI) analysis is a valuable tool in understanding complex genotype x environment interactions over years (Purchase *et al*., 2002). It also successfully determines stability of genotypes over environments and is therefore the ideal technique to identify superior wheat genotypes in the Free State in the presence of genotype x environment interaction. Since the AMMI model can summarize relationships and patterns of genotypes and environments successfully and separate patterns in the data from noise, there is a gain in accuracy and decisions are therefore improved. Crossa *et al.* (1991) reported that classification based on AMMI-adjusted data is a clearer illustration than a classification based on the original data.

Displacement along the abscissa reveals differences in main effects in the AMMI biplot of IPCA1. The displacement in the ordinate shows differences in the genotype x environment interaction. Genotypes with IPCA scores near to zero have little interaction with environments. Environments with IPCA scores close to zero give a poor discrimination amongst genotypes. Ranking of genotypes with IPCA scores close to zero is more reliable than ranking of genotypes with large positive or negative IPCA scores. Combinations of genotype and environment interaction with IPCA score of the same sign produce positive specific interaction effects, whereas combinations with opposite signs produce negative interaction effects (Crossa *et al.*, 1991).

4.2.3.4 Prediction efficiency

The eight genotypes in 11 locations were evaluated by using a minimum SDS sedimentation volume of 60ml/5g as a single criterion. This was compared to a combined selection set where the falling number should be above 360s, flour yield above 55%, flour protein content above 12%, SDS sedimentation above 60ml/5g and mixing development time between 1.8-3.2 minutes.

4.2.3.5 Correlation

Pearson correlation coefficients were derived from the ANOVA using Agrobase (2005).

4.2.3.6 Multiple stepwise regression

Multiple stepwise regression was used to determine the characteristic that contributes the most to the variation in SDS sedimentation, the dependent characteristic. The independent variables werekernel diameter, hardness index, protein content, mixing development time and thousand kernel mass. The independent variable in the elite generation was protein content, mixing development time, falling number, grain yield and flour yield. The coefficient of determination (R^2), expressed as a percentage, indicates the proportion of variation that can be explained by the relationship between X and Y.

4.2.3.7 Optimum number of locations and years

The effect of different numbers of replications, locations and years to determine the lowest theoretical variance as a mean was determined using the following equation by Rasmusson and Lambert (1961):

$$V_x = \frac{\delta^2_e}{rly} + \frac{\delta^2_{gly}}{ly} + \frac{\delta^2_{gl}}{l} + \frac{\delta^2_{gy}}{y}$$

With:

V_x - theoretical variance, r - replication, l - locations, y - years

Due to the negative variance component for genotype x location interaction, this formula was modified:

$$V_x = \frac{\delta^2_e}{rly} + \frac{\delta^2_{gly}}{ly} + \frac{\delta^2_{gy}}{y}$$

The least significant difference (LSD) was used as a measure of the precision, with the value being more precise at smaller values. With various combinations for replication, locations and year, the percentage LSD can be derived from the $V_{x,}$ with the formula:

$$LSD\% = \frac{(\text{t-value})*(2* V_x)*100}{\text{Grand Mean}}$$

4.3 RESULTS AND DISCUSSION

4.3.1 Analysis of variance

The influence of genotype on SDS sedimentation was highly significant when analysing the combined trials from 2004 to 2006 (Table 4.5a). The significant genotypic effect on SDS sedimentation indicates large differences in SDS sedimentation performance amongst the genotypes. Locations and years also had a highly significant influence on the SDS sedimentation performance of the genotypes as can be seen by the significant genotype x location, genotype x year and genotype x year x location interactions, indicating that there is an interaction between the environment and genotypes - genotypes performed differently at different locations in different years. It can therefore be concluded that testing of the genotypes needs to be conducted at different locations over different years for a reliable evaluation of SDS sedimentation performance of genotypes.

The combined analysis of variance of the SDS value (SV) for all the genotypes in trials conducted over years at different locations is presented in Table 4.5a. The influence of genotype was highly significant suggesting there were consistent differences in performance amongst the genotypes. Location and year also had an influence on the performance of the genotypes as can be seen by the significant genotype x location, genotype x year and genotype x year x location interactions. This indicates that the genotypes performed differently at different locations in different years. Therefore testing of the genotypes needs to be conducted at different locations over different years for a reliable evaluation of genotype SV performance.

The influence of genotype was highly significant in the combined analysis of variance of protein content (Table 4.5a). The influence of location, year, genotype x location, genotype x year and genotype x year x location were all highly significant, indicating that there is definitely an interaction between the environment and genotype with regard to protein content.

The combined analysis of variance for mixing development time is presented in Table 4.5b. The genotype main effect was highly significant, indicating that there were consistent differences in performance amongst genotypes. The environmental influence of genotype x location, genotype x year and genotype x location x year interactions were all highly significant, indicating that there was a large interaction between genotype and the environment for mixing development time and that genotypes performed differently at different locations in different years.

From the ANOVA in Table 4.5b it is evident that genotype, genotype x year, genotype x location and genotype x location x year had a highly significant influence on the variation in both, flour yield and grain yield.

Table 4.5a Analysis of variance for SDS sedimentation, SV and protein content

Source	df	SDS sedimentation (ml/5g)		SDS value		Protein content (%)	
		Sum of squares	Mean squares	Sum of squares	Mean squares	Sum of squares	Mean squares
Total	791	101332.21		543.14		759.21	
Replication	10	824.295	82.43**	6.80	0.68**	18.05	1.81**
Genotype	10	32222.50	3222.50**	199.06	19.91**	99.57	9.96**
Year	2	19756.62	9878.31**	35.56	17.78**	211.63	105.8**
Location	5	2641.63	528.33**	16.31	3.26**	59.18	11.84**
Genotype x year	20	17451.21	872.56**	94.15	4.71**	76.64	3.83**
Genotype x location	50	3769.50	75.39**	94.15	4.71**	21.66	0.43**
Year x location	10	8593.48	859.35**	58.22	5.82**	154.73	15.47**
Genotype x year x	100	8000.210	872.56**	42.70	0.43**	42.80	0.43**
Residual	584	548.00	80.00	58.26	0.10	74.95	0.13
Grand mean		63.72		4.70		13.57	
R-squared		0.92		0.89		0.90	
CV(%)		5.84		6.72		2.64	
b value		0.59		0.63		0.46	

** P ≤ 0.01

Table 4.5b Combined analysis of variance for mixing development time, flour yield and grain yield

Source	df	Mixing development time		Flour yield (%)		Grain yield (ton/ha)	
		Sum of squares	Mean squares	Sum of squares	Mean squares	Sum of squares	Mean squares
Total	791	255.56		24091.456		759.21	
Replication	10	1.82	0.18**	180.57	18.06**	18.053	1.81**
Genotype	10	143.71	14.37**	1555.83	155.58**	99.57	9.96**
Year	2	12.86	6.43**	16800.71	8400.36**	211.63	105.8**
Location	5	19.85	3.97**	659.28	131.86**	59.18	11.835**
Genotype x year	20	21.253	1.06**	938.70	46.94**	76.64	3.83**
Genotype x location	50	7.56	0.15**	572.69	11.45**	21.66	0.43**
Year x location	10	18.65	1.87**	863.51	86.35**	154.73	15.47**
Genotype x year x	100	13.75	0.14**	1016.95	46.94**	42.80	0.43**
Residual	584	16.12	0.03	1503.21	2.57**	74.950	0.13
Grand mean		2.42		60.78		13.57	
R-squared		0.94		0.94		0.90	
CV (%)		6.87		2.64		2.64	
b value		0.87		0.51		0.66	

**** P ≤ 0.01**

4.3.2 Means

Table 4.6 ranked the mean SDS sedimentation performance of the genotypes at six locations over three years. The SDS sedimentation of G3 was significantly higher than the rest of the genotypes. The SDS sedimentation of G4 and G2 was significantly lower than that of the rest of the genotypes. SDS sedimentation successfully discriminated between the closely related sister lines G5-G8. Komati, G1 and G6 were not significantly different from each other, neither were G2 and G4. It is in contrast with the report of Moonen *et al*. (1982) that although the SDS sedimentation was able to discriminate bread-making quality, it was unable to differentiate between subgroups.

SV successfully discriminated between all the lines, except for G3 and G5 (Table 4.6). Genotypes G8 and G9 were significantly different. The SV of Elands was significantly higher than that of the rest of the genotypes, followed by G5. The SV of G4 was significantly lower than that of the rest of the genotypes. Pickney *et al*. (1957) suggested that corrections for protein content be made by dividing the SDS sedimentation by the protein content to give a sedimentation ratio or specific value, but Atkins *et al*. (1965) concluded that the specific value did not show any advantage over the sedimentation value. This is in accordance with the findings of this study.

The protein content of G4 was significantly higher than that of the rest of the genotypes, while the protein content of G2, G5 and G7 were significantly lower than the rest of the genotypes, but not significantly different from each other (Table 4.6). This could be attributed to the fact that G5 and G7 are sister lines with identical pedigrees.

The mixing development time of G1 and Elands were significantly longer than that of the rest of the genotypes (Table 4.6). The mixing development time of G4 and G8 was significantly shorter than those of the rest of the genotypes. The discrimination ability of mixing development time was lower than that of SDS sedimentation, but it was less influenced by year and location which suggested that it would be beneficial to combine the two different traits as selection parameters in early generations.

The flour yield of Komati was significantly higher and the flour yield of G2 significantly lower than that of the rest of the genotypes (Table 4.6). The grain yield of G3 and G4 was significantly higher and the grain yield of Komati, G5 and G6 significantly lower than that of the rest of the genotypes (Table 4.10).

Table 4.6 Mean values of the parameters for the genotypes from 2004 to 2006

Genotype	SDS (ml/5g)	R	SV (%)	R	Protei (%)	R	Mixing development (min)	R	Flour (%)	R	Grain yield (tons/ha)	R
Elands	70.32b	2	5.29a	1	13.28e	8	2.98a	2	63.02b	2	2.82c	6
Komati	63.97e	8	4.57f	8	13.99b	2	2.69c	4	63.69a	1	2.60e	11
G1	64.11e	7	4.75e	7	13.48d	6	3.02a	1	60.70d	5	3.06ab	3
G2	52.28g	10	3.93h	10	13.23ef	9	2.83b	3	58.72f	11	3.02b	4
G3	71.40a	1	5.14b	3	13.87c	4	2.40f	7	60.36d	8	3.11a	2
G4	52.21g	11	3.71i	11	14.14a	1	1.89gh	10	61.28c	3	3.12a	1
G5	67.86c	4	5.16b	2	13.17f	10	2.58d	5	59.51e	9	2.61e	10
G6	64.51e	6	4.86d	6	13.28e	7	1.93g	9	60.44d	7	2.63e	9
G7	66.17d	5	5.04c	4	13.15f	11	1.94g	8	60.73d	4	2.68d	8
G8	58.68f	9	4.28g	9	13.84c	5	1.87h	11	60.62d	6	2.84c	5
G9	69.39b	3	5.01cd	5	13.88c	3	2.50e	6	59.48e	10	2.70d	7
Grand	63.72		4.70		13.57		2.42		60.79		2.84	
LSD	1.02		0.09		0.10		0.05		0.44		0.06	
CV	5.84		6.72		2.64		6.87		2.64		7.63	

Means followed by different letters are significantly different at P≤0.05

R – rank

4.3.3 Variance components

The estimates for the variance components of SDS sedimentation are presented in Table 4.7. The higher the variance component for the specific character, the larger the contribution to the total variation of the character. The contribution by genotype was 25.4%, genotype x location -0.3%, genotype x year 51.31%, genotype x location x year 12.85% and error 10.74% on the total variation in SDS sedimentation. The contribution by genotype was relatively high which makes effective selection for SDS sedimentation possible. The large genotype x year effect on performance in contrast with the genotype x location effect indicates that testing of genotypes across years may be more important than testing of genotypes across locations. The relatively low genotype x location x year (δ^2_{gly}=12.85%) contribution supports this view.

The estimates for the variance components of SV are presented in Table 4.7. The genotype contribution to the total variation in SV was similar to that in SDS sedimentation (only 2.6% difference). This small difference in genotypic effect suggest that SV may not effectively eliminate the effect of protein quantity (and protein content is highly influenced by the environment). If this is the case, an improved calculation to eliminate the protein quantity effect on SDS sedimentation should be developed, as was the aim of Greenaway *et al*. (1966). Loaf volume would most probably have to be included in this calculation, since this is the final "end-product" to successfully assess quality, as was the case with the study of Zeleny (1947). Another way to eliminate the effect of protein quantity on SDS sedimentation may be dividing the SDS sedimentation by dry gluten content, as suggested by Zhang *et al*. (2008). This would give an indication of the gluten quality and this matter needs further investigation. The similar genotypic effect on SDs sedimentation and SV may also suggest that protein quality, as measured by the SDS sedimentation, is even more susceptible to environmental influence than protein content (Graybosch *et al*., 1996), especially the percentage of protein present as gliadin and non-gluten proteins (Zhang *et al*., 2008). Although subunit composition (quality) is genetically fixed and is therefore unable to explain the portion of wheat quality variation that is dependent on environmental factors or genotype x environment interactions (O'Brien and Ronalds, 1983), the concentration (quantity) in which these subunits occur is highly influenced by the environment. The subunits of glutenin differ in their effect on wheat protein quality (Kadar and Moldovan, 2003). Although small, the genotype x year component of SV was positive (δ^2_{gl}=0.39) in contrast with a negative value for SDS sedimentation (δ^2_{gl}=-0.30). The influence of the genotype x location, genotype x location x year components on the variation in SV was more or less the same as SDS sedimentation.

The effect of variance components to the total variation in protein content is shown in Table 4.7. Similar to SDS sedimentation, the genotype x year component made the largest contribution to the total variation in flour protein content (49.59%). The large environmental influence (specifically genotype x year in this study) on protein content might explain the influence of environment on SDS sedimentation (Van Lill *et al*., 1995), probably by influencing changes in the contents of gluten proteins (Blumenthal *et al*., 1993). Several environmental factors might be responsible for the changes, for instance temperature (Blumenthal *et al*., 1991). Van Lill and Smith (1997) reported that the environment had a 18 times greater effect than genotype (E/G was calculated by dividing the mean squares of the environment by the mean squares of the genotype). The findings are in compliance with studies indicating that the environmental influences were generally large on protein content (Baenziger *et al*., 1985; Fowler and de la Roche, 1975b). Selection of superior quality genotypes solely on protein content could therefore be less efficient, as the large environmental effect on this value could give a less reliable reflection of the genotypic potential. This indicates that environment x line interactions could reduce effectiveness of early generation selection for quality characteristics under certain conditions (Kadar and Moldovan, 2003; Lebsock *et al*., 1964).

The genotype component of mixing development time was large (58.02%), 33.77% more than the genotype x year and 57.65% more than the genotype x location component (Table 4.7). The genotypic component was also 49.52% more than the genotype x location x year component. This suggests that the differences in mixing development time performance amongst genotypes over years and locations are very consistent. It can be concluded that mixing development time is also a very useful quality test to guide selection. It is recommended that SDS sedimentation, as a simpler and more cost effective technique of the two, should be performed to do selection on all the material in the early generations, and mixograph development time should be applied on the remaining genotypes after initial selection.

The relatively small influence of the genotype (16.47%) on flour yield suggests that the differences amongst genotypes were not consistent (Table 4.7). The large genotype x year effect indicates that the influence of year on flour yield of the different genotypes is large and differs from season to season. Since the year effect had the greatest influence on genotype performance, it is suggested that testing of genotypes across years may be more important than testing of genotypes across locations.

Genotype (21.82%), genotype x year (29.77%) and genotype x location x year (16.28%) contributed the most to the total variation in grain yield (Table 4.7). The large genotype x

year component suggests that the influence of year on grain yield of the different genotypes was large and differs from season to season. The interaction of genotypes with locations and years indicates that the genotypes performed differently at different locations in different years. Therefore the testing of the genotypes needs to be conducted at different locations in different years. Since the genotype x location effect had the least influence on genotype performance, testing of genotypes across years may be more important than testing of genotypes across locations, but the high genotype x location x year variance component requires testing across years and locations because the locations are different in different years.

Table 4.7 Estimated variance components and their relative contribution to total variation in the different parameters

Variance component	SDS sedimentation	SV	Protein content	Mixing development time	Flour yield	Grain yield
Genotype (δ^2_g)	32.70	0.21	0.09	0.19	1.41	0.03
Percentage of total variance	25.4	28.01	14.86	58.02	16.47	21.82
Genotype x location (δ^2_{gl})	-0.38	0.002	0.0004	0.001	0.63	0.002
Percentage of total variance	-0.30	0.39	0.07	0.37	7.33	1.21
Genotype x year (δ^2_{gy})	66.05	0.36	0.28	0.077	3.58	0.05
Percentage of total variance	51.31	47.43	49.59	24.25	41.99	29.77
Genotype x location x year	16.54	0.08	0.08	0.03	0.34	0.02
Percentage of total variance	12.85	10.87	13.12	8.56	4.02	16.28
Plot error (δ^2_e)	13.82	0.2	0.13	0.03	2.57	0.05
Percentage of total variance	10.74	13.30	22.37	8.80	30.18	30.92
Total	100	100	100	100	100	100

M - mean square

4.3.4 Canonical variate analysis

The CVA analysis was done on the averages of the four individual replications. In the CVA of the trials from 2004 to 2006 over eight locations there were 11 cultivars groups. The first two discriminant latent vectors and correlation coefficients of parameters and of cultivars for the quality parameters are presented in Tables 4.13-4.14.

At least one of the latent roots has to be greater than 1 in order for the analysis to be significant. In this case the latent root 1 was 1.90 (Table 4.13), which proves this analysis meaningful, and clearly differentiated among entries.

The first two canonical variates (CV1 and CV2) accounted for 81.45 of the variation (Table 4.14). The parameters discriminating between the genotypes were mixing development time, SV (r=0.988) and SDS sedimentation (r=0.887) (Table 4.14) as these correlated the strongest with the CV scores. Mixing development time strongly correlated (r=0.905) with the CVA1 score, while SDS sedimentation and SV strongly correlated with the CV2 score. The canonical variate means for SDS sedimentation and SV were negative and for mixing development time positive, thus it clearly contrasts these parameters.

The canonical variate means for each line (scores for CVA1 and CVA2) were plotted in Figure 4.1, illustrating the groupings of genotypes for the different parameters that differentiated them effectively over locations and three years. The separation between the genotypes is clear on both the CV1 and CV 2 axis, since it accounted for such a large amount of the variation. Genotypes further apart are dissimilar with regard to the variates that discriminate between them. Genotypes G7, G3 and G6 had short mixing development time and high SDS sedimentation and SV. Genotype G9 had an average mixing development time and high SDS sedimentation and SV. Genotypes G8 and G4 had low SDS sedimentation and SV and short mixing development time. Genotype G2 and Komati had a long mixing development time and low SDS sedimentation and SV. Elands, G5 and G1 had long mixing development times and high SDS sedimentation and SV.

The genotypes with the highest SDS sedimentation were Elands, G3, G5 and G9. These were also the genotypes with the highest SDS sedimentation in the ANOVA (Table 4.6). The genotype with the highest SV was G8, but G8 ranked low in the ANOVA (Table 4.6). The genotype with the longest mixing development time were G1 and G2. It is also the genotypes ranking high in the ANOVA of mixing development time in Table 4.6.

A 95% confidence circle indicated significant differences (Figure 4.2). The radius of the circle is given by the square root of the 95% point of chi-square variable. It makes sense that genotypes (G6 and G7) were close together, since they are sister lines, however genotypes G5 and G8 also have identical pedigrees to G6 and G7, but performed differently. Genotypes G9 and G3 and G9 and G5 were closely together despite the fact that their pedigrees are different. It can therefore be concluded that SDS sedimentation will effectively discriminate between closely related advance materials in a breeding program.

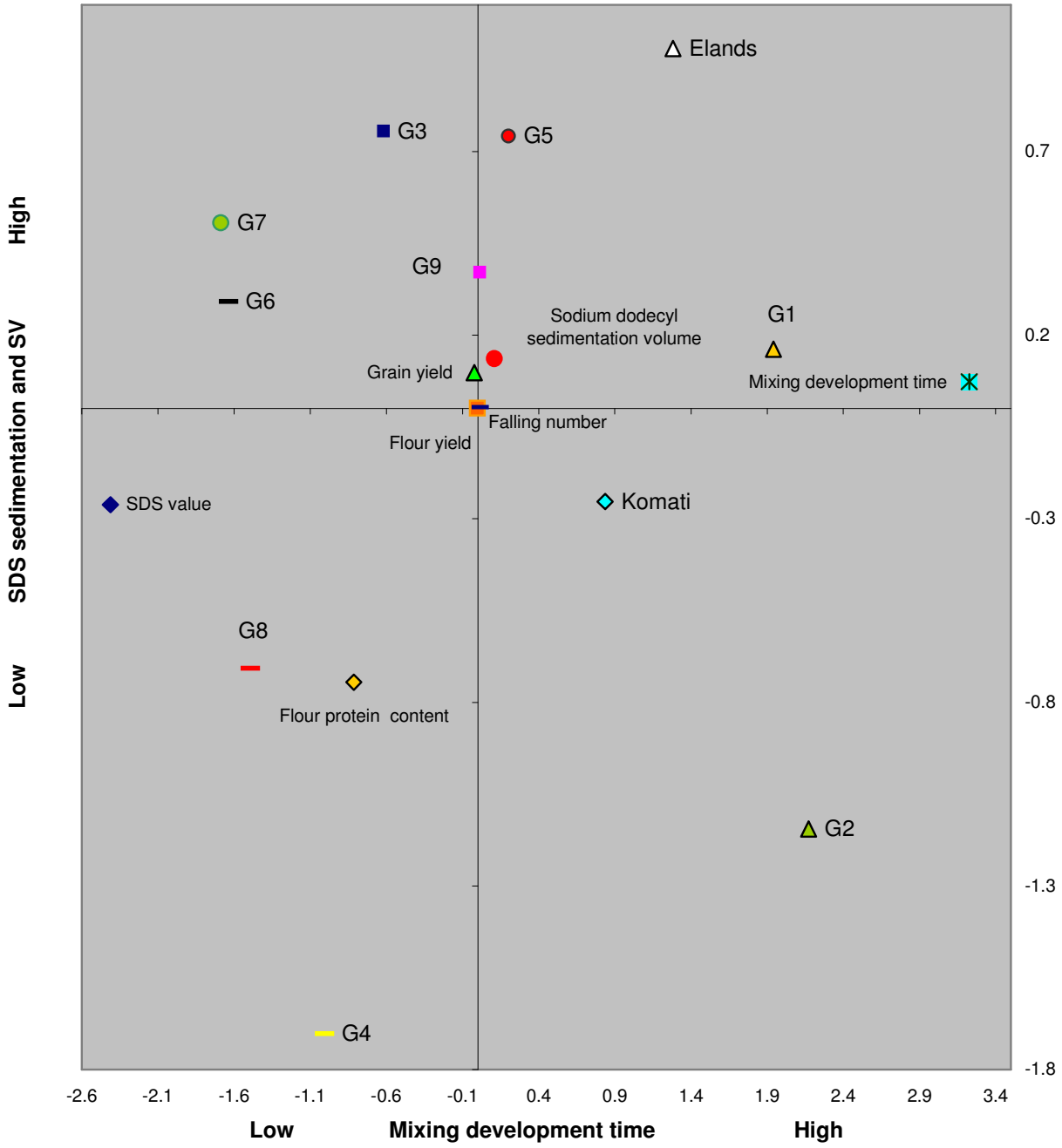


Fig. 4.1 Grouping of parameters in years 2004 to 2006 and eight locations in the Eastern Free State, using mean values

Table 4.13 Latent vectors as an indication of the correlation between the parameters

	X-axis	Y-axis			
	CV1	CV2	CV3	CV4	CV5
Latent roots	1.90	0.70	0.34	0.16	0.05
Variation explained (%)	59.55	21.90	10.50	5.03	1.64
SDS sedimentation (ml/5g)	0.109	0.136	0.488	0.996	0.899
Falling number (s)	-0.003	0.001	-0.003	-0.009	0.000
Flour yield (%)	0.016	0.004	0.019	-0.079	0.122
Flour protein content (%)	-0.814	-0.745	-0.769	-4.818	-4.299
Mixing development time (minutes)	3.226	0.073	-0.081	-0.427	-0.276
Yield (tons/ha)	-0.024	0.098	0.846	-0.230	-0.709
SV	-2.413	-0.262	-6.146	-	-

CVA1 – First canonical variate, CVA2 – Second canonical variate

Table 4.14 Correlation matrix between the variables and CVA scores

	SDS sedimentation	Falling number	Flour yield	Flour protein	Mixing development	Grain yield	SV
CVA1	-0.056	-0.079	0.001	-0.054	*0.905	0.044	-0.052
CVA2	*0.887	0.063	0.044	-0.269	*0.359	-0.137	*0.988
CVA3	0.297	-0.104	0.091	0.701	0.094	0.155	0.025

Coefficient significant at P≤0.05

Table 4.15 Latent vectors as an indication of the correlation between the genotypes

	X-axis	Y-axis			
	CV1	CV2	CV3	CV4	CV5
Elands	1.2816	0.9808	-0.1647	-0.6120	0.0188
Komati	0.8364	-0.2535	0.4093	-0.3778	0.5376
G1	1.9413	0.1615	0.0768	-0.1542	-0.1847
G2	2.1719	-1.1452	-0.5164	0.7172	0.0483
G3	-0.6199	0.7557	1.1881	0.3465	-0.0837
G4	-1.0073	-1.7031	0.3122	-0.4442	-0.1880
G5	0.2024	0.7425	-0.7037	-0.1210	-0.2966
G6	-1.6369	0.2917	-0.5049	0.2634	0.2597
G7	-1.6872	0.5057	-0.6401	0.1188	0.0843
G8	-1.4943	-0.7077	-0.0808	-0.1739	-0.1117

G9	0.0121	0.3715	0.6242	0.4371	-0.0840

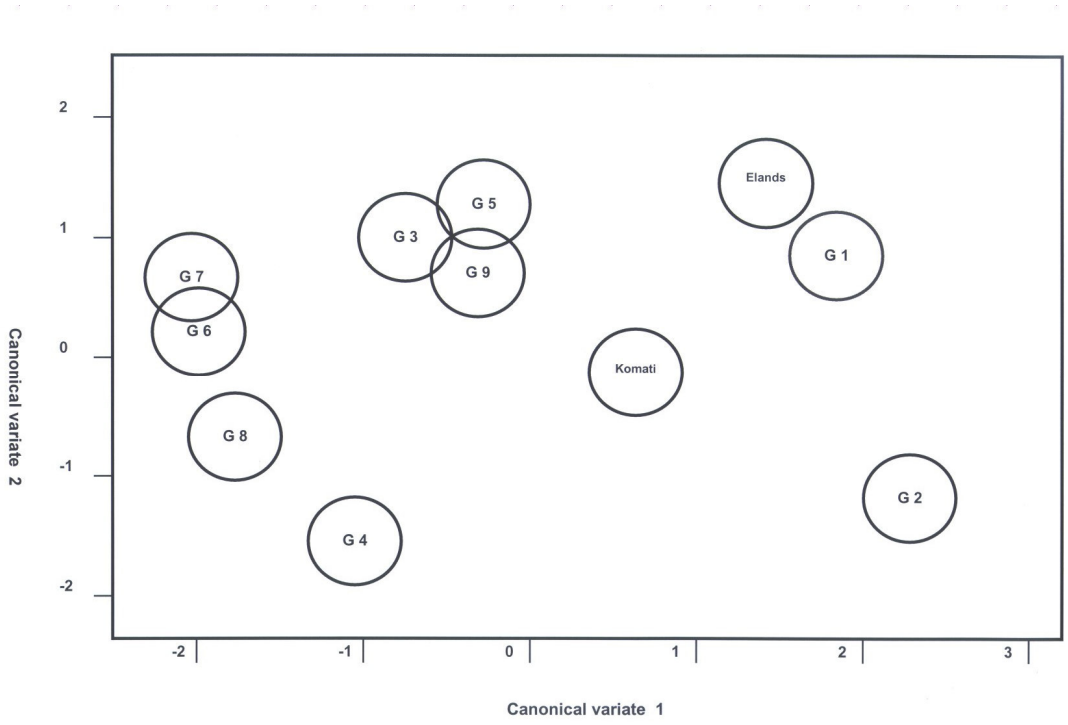


Figure 4.2 Canonical variate analyses of the genotypes from 2004 to 2006

4.3.5 Additive Main effects and Multiplicative Interaction

The AMMI analysis is a valuable tool in understanding complex genotype x environment interactions over years and determining the adaptation of genotypes over environments by graphically illustrating it in a biplot to show both main and interaction effects (Purchase, 1997; Purchase *et al*., 2002). The additive main effects and multiplicative interaction of the genotypes are presented in Figure 4.3.

SDS sedimentation

The scores for genotypes and locations are plotted in Figure 4.3. The IPCA axis 1 explained 50.94% of the variation, IPCA axis 2 21.53%; IPCA axis 3 9.96% and IPCA axis 4 explained 8% of the variation. Therefore 90.42% of the total variation was explained by the first four IPCA axes (Table 4.17). Since the first IPCA axis explains such a large amount of the variation, only the biplot graph of this axis is presented (Figure 4.3).

The further away from zero the IPCA score for the locations, the more interaction the environment has with the genotypes at that location and the more difficult it will be to select genotypes adapted to the specific location. Meets 2006, Kransfontein 2006, Kransfontein 2004, Meets 2004, Bethlehem 1 2004 and Bethlehem 2 2004 were such locations. Meets 2005, Kransfontein 2005, Clarens 2005, Bethlehem 2 2005 and Bethlehem 1 2005 were the locations where the genotypes had the smallest interaction with the environment and where the selection of adapted genotypes would be most efficient.

The effect of locations was not consistent from year to year. The locations in a single year were broadly grouped together, indicating a large season influence. If looking at the locations deviating –2 or 2 IPCA scores from the 0 axis, the locations in 2004 were clearly more unstable (Figure 4.3). This could be explained by the low rainfall and stress conditions that prevailed during 2004.

Within the genotypes, six groups could broadly be identified (Figure 4.3). Group 1 consisted of G8, group 2 of G6, G7 and G5, group 3 of G4, group 4 of G1and G9, group 5 with Elands, Komati and G3 and group 6 of G2.

Genotypes in groups 2, 4 and 5 had a good adaptation, whereas groups 1, 3, and 6 had poor adaptation. The influence of the environment on genotypes has to be taken into account, for it gives an indication of stability of a genotype. By selecting genotypes with high stability, more reliable performance can be expected. Groups 1, 2, 5 and 6 were unstable and less suitable to select from. Groups 3 and 4 had the most stable genotypes. Therefore, selecting from these

groups will result in a high predictive selection. Group 3 was poorly adapted though, and selecting for this group will result in negative genetic advance. Since group 4 (G1 and G9) is well adapted with a high stability, these are the genotypes that should be included in the selection program.

By selecting environments that successfully discriminate between genotypes and environments that produce consistent data, the breeding program's effectiveness can be increased. Since locations Kransfontein 2006, Meets 2006, Bethlehem 1 2004, Kransfontein 2004, Bethlehem 2 2004 and Bethlehem 2 2005 discriminated most effectively between genotypes, and locations Clarens 2005 and Bethlehem 2 were reliable and stable locations, their inclusion in the program is essential.

Table 4.16 AMMI analysis of variance of SDS sedimentation of the genotypes

Source	df	Sum of	Mean squares	Significance
Total	791	101650.51		
Locations	17	30957.19	1821.01	**
Replications x Locations	54	824.86	15.26	
Genotypes	10	32307.68	3230.76	**
G x E interaction	170	29482.14	173.42	**
IPCA 1	26	15016.85	577.57	**
IPCA 2	24	6346.36	264.43	**
IPCA 3	22	2935.39	133.45	**
IPCA 4	20	2358.39	117.92	**
IPCA 5	18	1227.94	68.22	**
IPCA 6	16	647.76	41.11	**
IPCA 7	14	468.48	33.46	
IPCA 8	12	229.27	19.11	
IPCA 9	10	158.90	15.89	
IPCA 10	8	82.26	10.28	
Residual	540	8079.64	14.92	
Grand mean = 63.85		**CV = 6.06**		
R – squared = 0.92				

**** P≤ 0.01**

Table 4.17 Eigenvalues and contribution to the total variance in SDS sedimentation

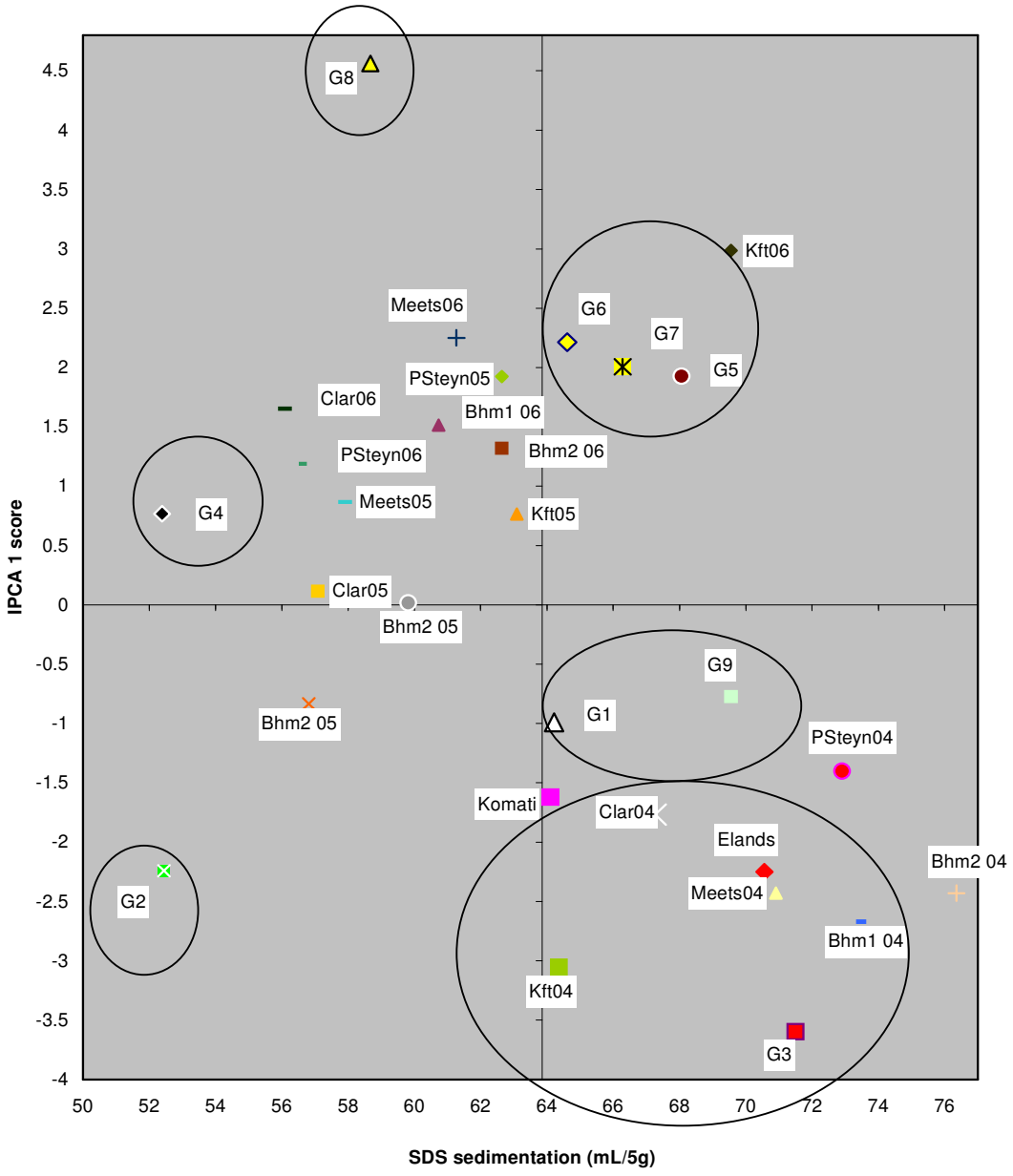
	Eigenvalue	% Genotype x environment	Cumulative %
1	3754.21	50.94	50.94
2	1586.59	21.53	72.46
3	733.98	9.96	82.42
4	589.60	8.00	90.42
5	306.99	4.17	94.58
6	164.44	2.23	96.82
7	117.12	1.59	98.40
8	57.32	0.78	99.18
9	39.73	0.54	99.72
10	20.57	0.28	100.00
Genetic variance (genotype) = 42.46			**STD error = 18.32**
Genetic variance (genotype x environment) = 39.62			**STD error = 4.68**

Table 4.18 Principal component analysis (IPCA 1) scores plotted against the SDS sedimentation of the genotypes

	IPCA 1 Score	Mean
Elands	-2.25	70.56
Komati	-1.62	64.11
G1	-0.99	64.22
G2	-2.24	52.44
G3	-3.60	71.50
G4	0.77	52.39
G5	1.93	68.06
G6	2.21	64.61
G7	2.00	66.28
G8	4.56	58.67
G9	-0.78	59.56

Table 4.19 Principal component analysis (IPCA 1) scores plotted against the SDS sedimentation for locations

	IPCA 1 Score	Mean
Clarens 2004	-1.77	67.27
Bethlehem 1 2004	-2.67	73.36
Bethlehem 2 2004	-2.43	76.36
Meets 2004	-2.43	70.91
Petrus Steyn 2004	-1.40	72.91
Kransfontein 2004	-3.10	64.36
Clarens 2005	0.12	57.09
Bethlehem 1 2005	0.02	59.82
Bethlehem 2 2005	-0.84	56.82
Meets 2005	0.87	57.91
Petrus Steyn 2005	1.92	62.64
Kransfontein 2005	0.76	63.09
Clarens 2006	1.65	56.09
Bethlehem 1 2006	1.32	62.64
Bethlehem 2 2006	1.51	60.73
Meets 2006	2.25	61.27
Petrus Steyn 2006	1.19	56.55
Kransfontein 2006	2.99	69.55



Key: Clar = Clarens, Bhm1 = Bethlehem 1, Bhm2 = Bethlehem 2, Cloc = Clocolan, Kransft = Kransfontein, Psteyn = Petrus Steyn

Figure 4.3 AMMI biplot of the genotypes and environment interaction of principal component analysis (IPCA 1) scores plotted against the SDS sedimentation for the genotypes for three years at six locations

SV

The IPCA axis 1 explained 51.68% of the variation, IPCA axis 2 23.07%; IPCA axis 3 7.92% and IPCA axis 4 explained 6.42% of the variation (Table 4.21). Therefore the first four IPCA axes explained 89.02% of the total variation. Since the first IPCA axis explains such a large amount of the variation, only the biplot graph of this axis was presented (Figure 4.4).

Meets 2006, Kransfontein 2006, Kransfontein 2004, Meets 2004, Bethlehem 1 2004 and Bethlehem 2 2004 were such locations. At Meets 2005, Kransfontein 2005, Clarens 2005, Bethlehem 2 2005 and Bethlehem 1 2005 locations the genotypes had the smallest interaction with the environment and this is where the selection of adapted genotypes will be more efficient.

The effect of locations was basically the same for SDS sedimentation and SV. The genotypes grouped into the same six groups that were evident in SDS sedimentation, but the grouping of SV was a mirror image of the grouping of SDS sedimentation volume. The same genotypes grouped together for SDS sedimentation and SV adaptability, only inversely. The same genotypes grouped together for SDS sedimentation and SV adaptability (Figures 4.3 an 4.4).

Since locations Bethlehem 1 2004, Clarens 2004, Bethlehem 2 2005 and Bethlehem 1 2006 discriminated the most effectively between genotypes and locations Clarens 2005, Petrus Steyn 2005, Kransfontein 2005 and Meets 2005 were reliable and stable locations, their inclusion in the a program evaluating SDS sedimentation in the Eastern Free State, South Africa is essential.

Table 4.20 AMMI analysis of variance in SV of the genotypes

Source	df	Sum of squares	Mean squares	Significance
Total	791	534.16		
Locations	17	110.08	6.48	**
Replications x Locations	54	6.80	0.13	
Genotypes	10	199.07	19.91	**
G x E interaction	170	159.95	0.94	**
IPCA 1	26	82.67	3.18	**
IPCA 2	24	36.90	1.54	**
IPCA 3	22	12.67	0.58	**
IPCA 4	20	10.28	0.51	**
IPCA 5	18	7.75	0.43	**
IPCA 6	16	4.49	0.28	**
IPCA 7	14	2.05	0.15	
IPCA 8	12	1.65	0.14	
IPCA 9	10	1.02	0.10	
IPCA 10	8	0.49	0.06	
Residual	540	58.26	0.12	
Grand mean = 4.70		**CV = 6.99**		
R – squared = 0.89				

****P ≤ 0.01**

Table 4.21 Eigenvalues and contribution to the total variance in SV

	Eigenvalue	% Genotype x environment explained	Cumulative %
1	20.67	51.68	51.68
2	9.22	23.07	74.75
3	3.16	7.92	82.67
4	2.57	6.42	89.09
5	1.94	4.84	93.94
6	1.22	2.81	96.75
7	0.51	1.28	98.03
8	0.41	1.03	99.06
9	0.25	0.64	99.70
10	0.12	0.30	100.00
Genetic variance (genotype) = 0.263			**STD error = 0.11**

Genetic variance (genotype x environment) = 0.208	STD error = 0.03

Table 4.22 Principal component analysis (IPCA 1) scores plotted against the SV for intermediate elite genotypes

Genotype	IPCA 1 score	Mean
Elands	0.30	5.29
Komati	0.24	4.57
G1	0.25	4.75
G2	0.98	3.93
G3	0.94	5.14
G4	-0.38	3.71
G5	-0.43	5.16
G6	-0.36	4.86
G7	-0.31	5.04
G8	-1.39	4.28
G9	0.14	5.01

Table 4.23 Principal component analysis (IPCA 1) scores plotted against the SV for locations

	IPCA 1 score	Mean
Clarens 2004	0.56	4.61
Bethlehem 1 2004	0.62	5.06
Bethlehem 2 2004	0.82	5.53
Meets 2004	0.54	4.59
Petrus Steyn 2004	0.42	5.49
Kransfontein 2004	0.88	4.62
Clarens 2005	-0.10	4.51
Bethlehem 1 2005	0.09	4.22
Bethlehem 2 2005	-0.08	4.45
Meets 2005	-0.13	4.27
Petrus Steyn 2005	-0.69	4.49
Kransfontein 2005	-0.52	4.89
Clarens 2006	-0.26	4.47
Bethlehem 1 2006	-0.27	4.73
Bethlehem 2 2006	-0.34	4.44
Meets 2006	-0.56	4.71
Petrus Steyn 2006	-0.32	4.40
Kransfontein 2006	-0.70	5.16

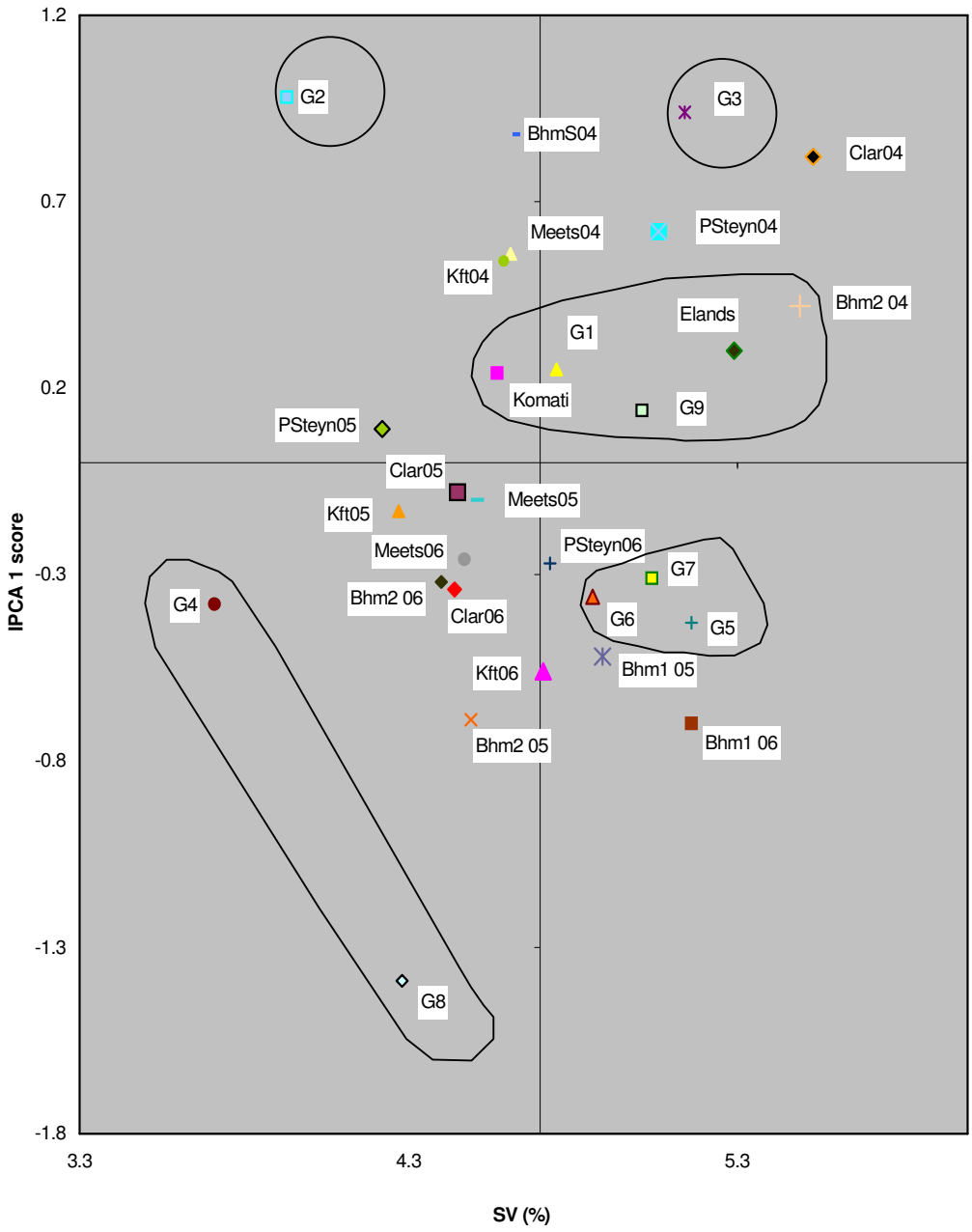


Figure 4.4 AMMI biplot of the genotype with environment interaction of principal component analysis (IPCA 1) scores plotted against the SV of the genotypes for three years at six locations

4.3.6 Prediction efficiency

To evaluate the effectiveness of predicting the overall quality potential of a line by using its SDS sedimentation only, the eight genotypes in 11 locations were evaluated by using a minimum SDS sedimentation of 60ml/5g as a single criterion. An amount of 62.12% of the samples for all the genotypes over all the locations would have been selected on their SDS sedimentation alone, whereas 41.67% of the samples would have been selected by considering all the available quality data. SDS sedimentation thus gives a fairly good indication, with the exception of a few, of the overall quality of a genotype for South African quality standards.

4.3.7 Correlation

As expected, the association between SDS sedimentation and protein content was highly significant ($r=0.44$ at $P\leq0.01$). However, the correlation varied greatly from genotype to genotype, which is in accordance with the findings of Silvela *et al.* (1993). The correlation also varied according to the locality and the season (data not presented), which is in compliance with(Bushuk *et al*., 1969; Moonen *et al*., 1982; Dick and Quick, 1983; Lorenzo and Kronstad, 1987; de Villiers and Laubscher, 1995; Carter *et al*., 1999; Cubadda *et al*., 2007; Morris *et al*., 2007).

The relationship between SDS sedimentation and mixing development time ($r=0.37$, $P\leq0.01$) was highly significant (Table 4.24). Selection of higher SDS sedimentation may result in stronger dough characteristics in the following generations of certain genotypes, which is not always suitable in all breeding programs, since dough of a medium strength is preferred in the South African market. Van Lill *et al.* (1995) also warned against the breeding of excessively strong dough.

There was no significant relationship between SDS sedimentation and flour yield, and SDS sedimentation and falling number. The negative correlation between SDS sedimentation and grain yield was highly significant ($r=-0.42$ at $P\leq0.01$). This could possibly be attributed to the influence of protein content, because it is well-known that a negative association exists between protein content and grain yield and this was also evident in this study ($r=-0.59$, $P\leq0.01$). It can be concluded that to do selection on SDS sedimentation only could ultimately lead to a stabilization of yield in subsequent generations.

Table 4.24 Correlation matrix including all the genotypes over three years from eight locations in the Eastern Free State

	SDS	FN	FPC	MDT	FLY	YLD
Falling number (FN)	-0.13					
Flour protein content (FPC)	0.44 **	0.10				
Mixing development time (MDT)	0.37 **	0.01	0.08			
Flour yield (FLY)	-0.15	0.37 **	0.08	0.02		
Grain yield (YLD)	-0.42 **	-0.17	-0.59 **	0.02	-0.33 **	

**** P≤0.01, * P≤0.05**

4.3.8 Stepwise multiple regression

Stepwise multiple regressions were used to determine the character that contributes the most for the variation in SDS sedimentation, the dependent characteristic. The independent variable in the elite generation was protein content, mixing development time, falling number, grain yield and flour yield. The coefficient of determination (R^2), expressed as a percentage, indicates the proportion of variation that can be explained by the relationship between X and Y. Grain yield and mixing development time explained the most to SDS sedimentation. It was possible to explain 13% and 9% of the variation in SDS sedimentation due to grain yield and mixing development time. The other characteristics made only minor contributions and were of insignificant value in predicting variation in SDS sedimentation.

Table 4.25 Adjusted R for all the traits in the model, explaining the variation in SDS sedimentation in the elite material (P≤0.05) at the eight localities over three years

Locality	Action	Variable	R^2	Mean square error	Model p-level
0	Unchanged		0.00	11.38	0.00
1	Added	YLD	0.13	10.60	0.00
2	Added	MDT	0.22	10.06	0.02
3	Unchanged		0.22	10.06	0.02

4.3.9 Optimum number of locations and years

Due to the negative variance component for genotype x locality interaction it was only possible to calculate the optimum number of years and replications.

The combination of years, replications and locations that results in the lowest $V_{x,}$ is ideal, but the practicality of increasing each factor must be taken into consideration. The factor that is more effective than another in reducing V_x will determine its relative importance. Locations are considered, because the number of locations has large financial implications for a breeding program. Secondly, years were considered because the number of years will have large implications for a breeding program, since it is the goal of any program to obtain information in the shortest possible time.

Firstly, years and thereafter locations were manipulated to determine the optimum number of each to be included in the program for effective SDS sedimentation selection. Figure 4.5 and 4.6 represent the LSD% plotted for different number of locations and years respectively. From Figure 4.5, it is clear that the rate of gain in precision decreases as the number of locations increases and it is clear that one to two locations is adequate to perform effective SDS sedimentation selection in early generations. A further increase of locations will result in a very small increase in precision, but would have large practical and financial implications and is therefore not recommended.

Figure 4.6 represents the effect of a different number of years on the gain in trial precision. An increase in the number of years will dramatically increase the gain in precision of the trial mean. Significant trial mean precision is gained by increasing the number of years from one to three and then to five, although the size of the precision increment decreases. It is therefore recommended that four to five years is essential for reliable SDS sedimentation selection. This can be done by selecting in the F2 to F5 generations in a breeding program, which will result in a decrease in variation and an improvement in SDS sedimentation stability. This will result in a drastic decrease in the effect of genotype x environmental interaction, which will make further selection later in the breeding program for SDS sedimentation selection unnecessary.

From Figure 4.7, it is clear that although an increase in both the number of locations and number of years increases the precision of the trial mean, the increase of years results in a far more effective increase in precision than an increase of locations. In order to increase the precision of SDS sedimentation selection, an increase in the number of years is therefore recommended, rather than an increase in the number of locations.

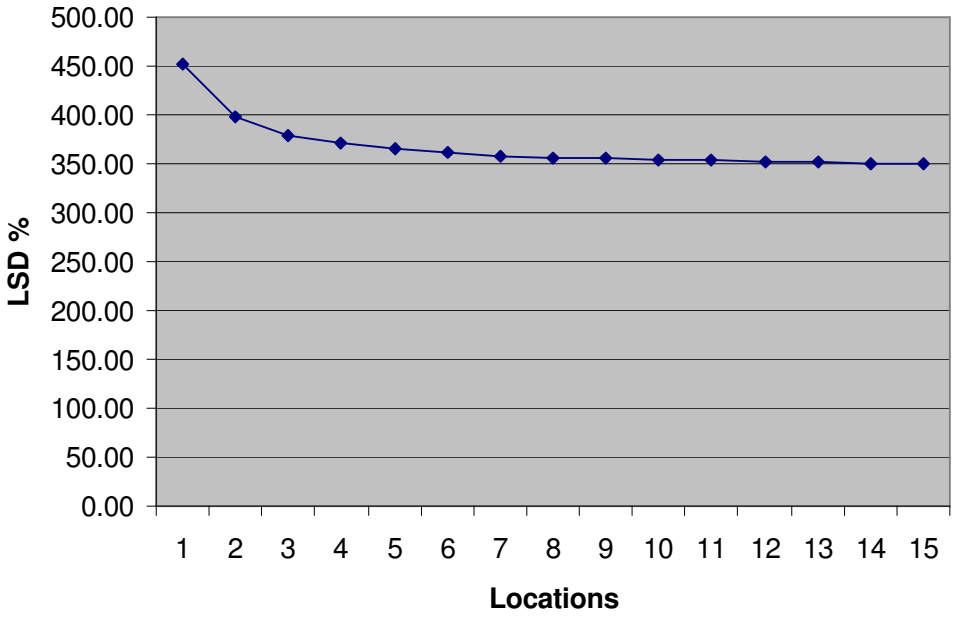


Figure 4.5 Effect of number of locations and replications on trial mean precision

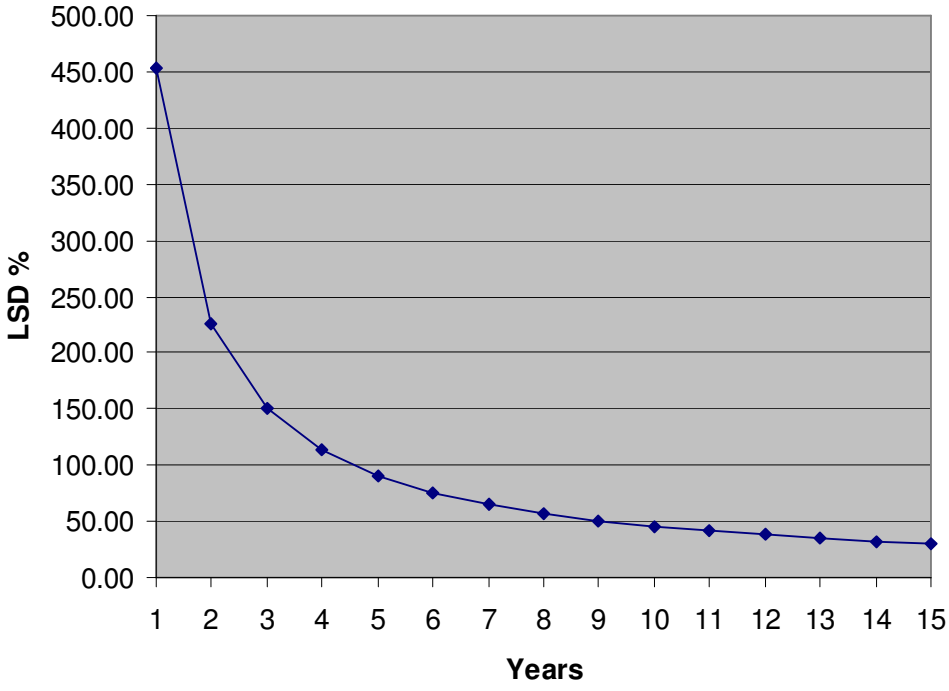


Figure 4.6 Effect of number of locations and replications on trial mean precision

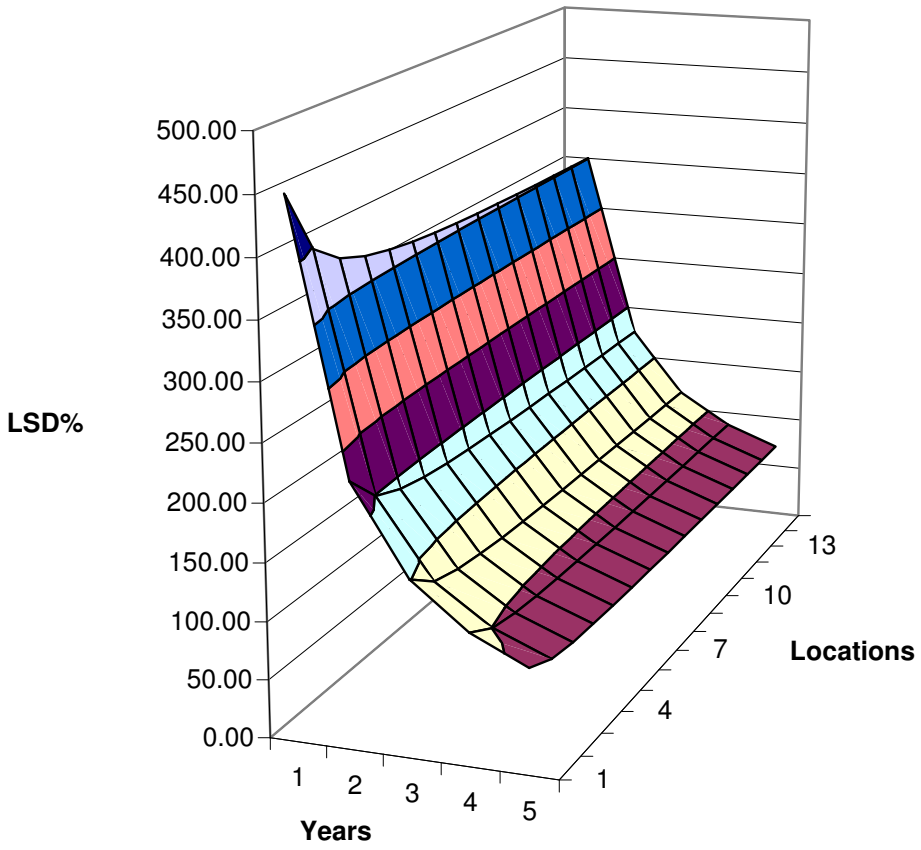


Figure 4.7 Effect of number of locations and replications on trial mean precision

4.4 CONCLUSIONS

The objective of this study was firstly to establish the significance of genotype x environment interaction on SDS sedimentation, secondly to determine the discrimination ability of SDS sedimentation, thirdly to determine the optimal number of locations required for efficient SDS sedimentation testing and fourthly to determine the relationship of SDS sedimentation with other quality traits and grain yield.

The ANOVA indicated that the genotype contribution was highly significant, which was in accordance with other reports in the literature. The environmental x genotype interaction was higher (especially the genotype x year interaction) and the genotypic component smaller than reported in literature. This could reduce the effectiveness of SDS sedimentation selection.

The genotype x location, genotype x year and genotype x year x location interactions were significant for SDS sedimentation. The estimated variance components showed that $\delta^2_{g,}$ $\delta^2_{gl,}$ δ^2_{gy} and δ^2_{gly} contributed 25.40, -0.38, 66.05, 16.54 percent to the total phenotypic variance respectively. The genotypic contribution of 25.40% is large enough to allow effective selection for SDS sedimentation.

It is clear that the year had a large influence on SDS sedimentation and trials would have to be conducted over years for assessment of the true potential of the genotypes. A possible explanation for the large year influence in this study could be the contrasting weather conditions (rainfall) that prevailed in the three years. The large genotype x year component suggests that testing of genotypes across years may be more important than testing of genotypes across locations.

The genotype contribution to the total variation in SDS sedimentation was 25.4%, while the genotype contribution in the total variation in mixing development time was 58.02%. Compared to mixing development time, SDS sedimentation appeared to be more subjected to environmental effects. This was supported by Van Lill *et al*. (1995). Mixing development time may therefore be a more reliable parameter than SDS sedimentation in situations where trials over locations and years are not possible, ora combination of both sedimentation and MDT to avoid selecting overlystron lines can be considered.

It was possible to effectively discriminate between genotypes in spite of their similar backgrounds. The genotypes with the highest means SDS sedimentation were Elands, G3, G5 and G9. These were also the genotypes with the highest SV. The genotype with the highest mean SV was G8. The genotype with the highest protein content was G4. The CVA confirms that SDS sedimentation (and mixing development time) discriminated effectively between cultivars. SV was not superior to SDS sedimentation and did not compensate for the influence of the environment on protein content.

In the AMMI it was clear that adaptability varied from year to year. Selection of high SDS sedimentation genotypes for a specific location based on only one year data is therefore not recommended.

Correlations in the overall correlation matrix did not necessarily exist in the correlations of individual populations or genotypes and visa versa. It was concluded that care must be taken when looking at the correlation of an individual population, because a single population might result in biased correlations, whereas the combined correlation matrix including multiple genotypes is more reflective of the general correlations in the population. The correlation between SDS sedimentation and protein content ($r=0.44$, $P\leq0.01$) was in accordance with reports in literature. The negative correlation between SDS sedimentation volume and grain yield can be explained by the dependence of SDS sedimentation on protein content and the negative association between protein content and yield.

The relationship between SDS sedimentation and mixing development time ($r=0.37$, $P\leq0.01$) was highly significant. Similarly, Fowler and De la Roche (1975a) also reported that SDS sedimentation reflected quantity of protein and rate of dough development, which is in accordance with the findings of this study. Selection of higher SDS sedimentation may result in stronger dough characteristics in the following generations of certain genotypes, which is not always suitable in all breeding programs, since dough of a medium strength is preferred in the South African market. It is therefore recommended to follow selection of SDS sedimentation up with mixing development time selection in the following year in early generations where limited seed and large numbers are limiting factors. In advanced generations where more seed is available and the number of genotypes is less, it is recommended to combine SDS sedimentation and mixing development time evaluation for selection.

Grain yield and mixing development time contributed the most to SDS sedimentation in the elite material. It is therefore clear that mixing development time had a great influence on SDS sedimentation or is related to the same aspect, presumably gluten.

Variance components indicated that the genotype x location x year (δ^2_{gly}=12.85%) component was relatively small, but highly significant. It can therefore be concluded that testing of genotypes across years may be more important and testing of early generation material for consecutive seasons at one or two locations is adequate for reliable evaluation.

CHAPTER 5

THE EFFECT OF SELECTION FOR SDS SEDIMENTATION IN THE F2 GENERATION ON THE QUALITY OF THE SUBSEQUENT F4 GENERATION

5.1 INTRODUCTION

Since a single reliable selection parameter in the identification of the most productive genotypes represents a crucial point in many wheat breeding programs, several studies have been conducted to evaluate the suitability of SDS sedimentation as such a parameter (McNeal *et al*., 1969; Briggs and Shebeski, 1971; Blackman and Gill, 1980; Dick and Quick, 1983; Fischer *et al*., 1989; Ayoub *et al*., 1993; Baik *et al*., 1994; De Villiers and Laubscher, 1995; Carter *et al*., 1999; Morris *et al*., 2007). SDS sedimentation (AACC International Approved Method 56-70) has long been used to determine the quality of wheat genotypes in breeding programs and has gained wide acceptance as a useful small-scale test in bread wheat breeding programs to predict baking quality (Carter *et al*., 1999).

Apart from eliminating obvious defects such as disease- and pest susceptible plants or families and plants or families with poor adaptability, early generation selection in the F2 and F3 stage has mostly been subjective. Amongst progeny of well-adapted parents, however, it is increasingly difficult to identify high potential genotypes. The South African system of cultivar release puts a lot of emphasis on high quality, therefore more reliance must be placed on expensive quality testing in later generations of the breeding program, which leads to the discarding of a high number of lines in later generations in the breeding program as a result of poor quality. It is therefore desirable to examine the value of SDS sedimentation as an early generation selection criterion.

Many selection studies have been conducted with fixed lines, which are not subjected to further gene segregation and often biased through selection to an unknown degree Moreover, far too often, final assessment is done in single rows, hill plots or small plots, all of which are unbordered and subject to confounding effects (Fischer, 1989). In both cases, relationships are not necessarily relevant to breeding programs aiming to improve the characteristic that is selected (Quail *et al*., 1989).

Although SDS sedimentation has been criticized for its dependence on protein quantity, it must be kept in mind that the plant breeder is primarily concerned with the classification of breeding lines into broad categories to enable elimination, consequently the interest is in the relative sedimentation categories (Dewey, 1963).

Fasoula and Zaragotas (1990) reported that interplant competition and soil heterogeneity are important factors reducing the effectiveness of selecting single plants for improvement of polygenic characters like yield and quality. Comparing neighbouring plants to a standard check evenly distributed throughout the trial can minimize the effect of soil heterogeneity. Wide interplant spacing (60cm) would supposedly reduce the impact of competition, although there seem different opinions on this matter (Bos, 1981).

The purpose of this study was:

1. To establish the significance of genotype x environment interaction on SDS sedimentation in the F4 generation of hard red winter wheat
2. To determine the efficiency of selection of SDS sedimentation in seven populations of individual F2 plants grown under optimal conditions
3. To determine the discrimination ability of SDS sedimentation in the F4 generation between and within populations
4. To compare the SDS sedimentation and SDS value
5. To study the relation of SDS sedimentation with other quality traits in the F4 generation
5. To determine which traits contribute the most to the variation in SDS sedimentation in the F4 generation by performing stepwise regression

5.2 MATERIAL AND METHODS

5.2.1 Plant material

Seed of eight crosses and two commercial cultivars (one serving as a check and one as an entry) were selected from the F1 generation planted under conditions which are a true reflection of the commercial program currently operating in Bethlehem, South Africa by the ARC-Small Grain Institute. The crosses were all hard red winter type genotypes with strong dough characteristics, except for population 1, which was soft white wheat. Soft white genotypes with high- and low protein content do exist, but remain unselected as bread wheat because it is utilized as animal feed in South Africa. Soft white wheat is thus almost never used as crossing parents, except in cases where it has favorable characteristics other than quality, for instance disease resistance.

The F1 seed were planted in 2005 to generate F2 seed. Two of the populations were discarded in the first year. Population 8 had poor agronomical adaptability and population 9 was discarded because of its susceptibility to Russian wheat aphid. Betta-DN was used as standard check. This is a direct selection of Klein Impacto – a line imported from CIMMYT and a hard red wheat commercial cultivar released in South Africa in 1995, with excellent

quality characteristics. The commercial cultivar, Elands, that was released in 1997 and exhibits excellent quality and yield characteristics, was also included for two reasons:

1. To evaluate the effectiveness of using a “moving mean percentage” design
2. To compare a population in which segregation is almost non-existent, to the other populations in which segregation is still occurring

Table 5.1 Pedigree and background of material used in this study

	Pedigree	Background
1	PF83144/3/F3.71/TRM//Vorona/Mica/Tisza1"132"	Low protein, white, soft population with relative short mixing time
2	KS82142/Cupe//X92-A-13-1	Hard red winter cross, unrelated to the rest of the populations
3	Betta-DN//Saulesku28/Tugela-DN	Reciprocal cross 1, hard red winter
4	Saulesku28/Tugela-DN//Betta-DN	Reciprocal cross 1, hard red winter
5	Betta-DN//661L-33/Tugela-DN	Hard red winter
6	Betta-DN//Tisza1/Elands	Hard red winter
7	Elands	Commercial dry land cultivar in SA
8	Betta-DN/X920750-A-13-1	Discarded: Poor agronomical characteristics
9	Saulesku//SA463/*Tugela (28)/4/AGRI/NAC//KAUZ/3/1D13.1/MLT	Discarded: Severely susceptible to Russian wheat aphid infection
Check	Betta-DN	Commercial dry land cultivar in SA

5.2.2 Environment

F2 and F3

The F2 and F3 trials were conducted in 2005 and 2006 at the Small Grain Centre near Bethlehem in the Free State (24˚14'S latitude, 28˚18'E longitude). Historically, the Bethlehem area is associated with optimal growing conditions for wheat, which in turn have been shown to reduce environmental effects and enhance the expression of genetic potential (Van Lill *et al.*, 1995). The experimental plots were located on a fertile site where a crop fallow system was applied. The soil was characterized as an Avalon form, Soetmelk series having an orthic A horizon followed by a yellow-brown apedal B and a soft plinthic B-horizon (Table 5.2).

F4

The F4 trial was conducted in 2007 at Bethlehem, Meets and Clarens, all which are locations with high production potential where a crop fallow system was applied (Table 5.3).

Table 5.2 Location characteristics for the 2005 and 2006 growing seasons

Location	**Bethlehem**
Latitude	2008
Longitude	2818
[Θ]Rain	204.8mm (2005 season)
Crop history	Monoculture production system

Table 5.3 Location characteristics for the 2007 growing season

Location	**Bethlehem**	**Clarens**	**Meets**
Latitude	28° 09'14''S	28° 23'53''S	28° 06'42''S
Longitude	28° 17'17''E	28° 25'48''E	28° 07'23''E
[Θ]Rain	120.83mm	130.79mm	66.00mm
Crop history	Crop-fallow	Crop-fallow	Crop-fallow

[Θ] Rain is documented as the precipitation during the period of anthesis +60 days

5.2.3 Experimental practices and selection

F2

The F1 seeds were hand-planted on the 5th of July 2005. All the generations were planted at a wide spacing of 45cm between plants. Rows were one metre in length. A 45 centimetre inter-row spacing was used. The standard check was planted every second row throughout the trial in order to take into account all variation that might exist due to environmental influences, for instance variation in soil, temperature and moisture. Production techniques such as planting date and fertilizer application were aimed at maximum yield and optimum performance conditions. All the trials received 3:2:1 (25) N.P.K fertilizer prior to planting, at a rate of 250 kg ha^{-1}. No pesticides or fungicides were applied during the season in the F2 generation to allow identification of plant and families susceptible to pests and diseases.

Single plants with superior agronomical appearance were selected and labelled in the F2 generation at growth stages 61-77 (Zadoks *et al.,* 1974) as it would have been done for normal selection in the commercial breeding program (Figure 5.1). The number of plants selected depended on the agronomic appearance of each cross, with a higher number being selected if the agronomic appearance was good. The selected plants were harvested on the 21st of December 2005, air-dried and hand-threshed individually prior to quality testing. SDS sedimentation and protein content of the single plants were determined. In order to achieve a clearer expression of genotypic differences, the value of each plant was expressed as a percentage of the two nearest neighbouring check plants (SDS percentage). Ranking in the progeny of seven different crosses in a winter wheat breeding program by using SDS sedimentation and SDS value, was compared. Selection was done in two different ways: In the first group, selection was done on single plants by taking the SDS sedimentation into account and selecting the single plant with the highest SDS sedimentation value (selected group). In the second group, selection was done randomly without taking SDS sedimentation in account, although the values were determined (unselected group). In the unselected group a single plant with good agronomical characteristics was selected as is the case in the present breeding program where only visual selection is done, although SDS sedimentation was scored.

F3

The F3 trial was conducted in 2006, also at the Small Grain Centre near Bethlehem in the Free State (24˚14'S latitude, 28˚18'E longitude). The F3 seed were hand-planted on the 12th of July 2006. An inter-plant spacing of 45cm was used. The rows were 45cm apart and one metre in length. Production techniques such as planting date and fertilizer application were aimed at maximum yield and optimum performance conditions. All the trials received 3:2:1 (25) N.P.K fertiliser prior to planting, at a rate of 250 kg ha^{-1}. No pesticides or fungicides

were applied during the season in the F3 generation to allow identification of plant and families susceptible to pests and diseases. The two groups, where SDS was taken into account (SDS) and SDS were not taken into account (USDS), of the seven populations were individually bulk harvested for each population at growth stages 61-77 (Zadoks *et al.,* 1974) on the 15th of December 2006 (F3). It was then air-dried and hand-threshed individually. In the F3 generation, no quality analysis was performed.

F4

Ten grams of each group (selected and unselected) of all the populations was planted as the F4 generation. The trials were planted with a Gaspardo planter on 17 and 18 July 2007 at Bethlehem, Meets and Clarens. A randomized complete block design with four replications was used. A wide inter –plant spacing of 45cm was used. Rows were one metre in length. An inter-row spacing of 35cm was used. Production techniques such as planting date and fertilizer application were aimed at maximum yield and optimum performance conditions. All the trials received 3:2:1 (25) N.P.K fertiliser prior to planting, at a rate of 250 kg ha^{-1}. The rows were harvested on the 19th and 20th of December 2007 in separate bags prior to quality testing. SDS sedimentation, protein content, mixing development time, hardness index, thousand kernel mass and kernel diameter of the single rows were determined.

Generation							Action	
F1							Self pollinate	
							5g selected	
↓								
F2								
							Planted:	Single rows
Bethlehem							Quality tests:	SDS sedimentation, protein content
							Harvested:	Single plants selected
↓								
F3							Selection:	Selected group: Selection taking SDS into account Unselected group: Selection not taking SDS in account
							Planted:	Single rows
Bethlehem							Harvested:	Single rows bulked
↓							Quality tests:	None
F4							Selection:	None
*selected	unselected	selected	unselected	selected	unselected		Planted:	Four replications of single rows at three locations
							Harvested:	Single rows bulked
Bethlehem		Meets		Clarence			Quality test:	SDS sedimentation, protein content, mixing development time, hardness index, thousand kernel mass, kernel diameter

***Selected – group where highest SDS sedimentation in the F2 generation was taken selected**

Unselected – group where SDS sedimentation in the F2 generation was not taken in account

Figure 5.1 Procedure followed from the F2 to F4 generation

5.2.4 Quality analysis

5.2.4.1 SDS sedimentation

The approved Method 56-70 was followed (AACC, 2000) with certain modifications. Sample weight and SDS concentration were standardized. A whole meal sample of 5g was used, because of the limited seed available from a single plant. The sample was ground with a Perten 3100 laboratory mill, containing a standard sieve of 0.8mm. The SDS sedimentation technique was performed within 5 hours of grinding, at a stable room temperature of 24°C. All the chemical solutions were made up the day before the experiments were conducted. The SDS sedimentation volume was recorded after 30 minutes. Results are reported as specific sedimentation volume (ml/5g).

5.2.4.2 Protein content

Grain protein content was determined by using near-infrared reflectance spectroscopy (Infra Analyser, 360, Bran & Luebbe, Hamburg; AACC, 2000).

5.2.4.3 Mixing development time

Mixing development time was determined according to AACC*54-40A with a 35g National Mixograph (National Mfg. Lincoln, Nebraska). The mixing development time over a period of 6 minutes was recorded.

5.2.4.4 Hardness index

Hardness was determined by using the SKCS AACC Method 55-31 with the SKCS model 4100 instrument.

5.2.4.5 Thousand kernel mass

Thousand kernel mass (g) was determined using a SKCS model 4100 instrument, where the AACC*55-31 Method (AACC, 2000) was used.

5.2.4.6 Kernel diameter

Kernel diameter was determined by using the SKCS AACC*55-31 method with the SKCS model 4100 instrument.

5.2.4.7 SDS percentage

SDS percentage was calculated by expressing the SDS sedimentation as a percentage of the mean of the two adjacent check plants.

5.2.5 Statistical analysis

5.2.5.1 Analysis of variance

For the F4 generation, the total variation of each trait was evaluated in three different environments with four replicates. The combined analysis of variance (ANOVA) was used to test the significance of cultivar, location and their interactions for all the quality characteristics. Analysis of variance was carried out to compare genotype means and differences by means of the least significant difference (LSD at $P \leq 0.05$). Agrobase Generation II (Agrobase, 2005) was utilized to perform the analysis of variance and the regression.

5.2.5.2 Canonical variate analysis

Canonical variate analysis was used to determine which characteristics discriminate most between the cultivars. In this study, the variates represent the quality characteristics that were measured on the F4 wheat populations over three different environments in the Eastern Free State. The data were analysed, using GenStat (2000).

5.2.5.3 Additive main effects and multiplicative interaction

Additive main effects and multiplicative interactions were calculated by using the Agrobase (2005) program.

5.2.5.4 Correlation

Pearson correlation coefficients were derived from the ANOVA using Agrobase (2005).

5.2.5.5 Multiple stepwise regression

Multiple stepwise regression was used to determine the character that contributes the most to the variation in SDS sedimentation volume, the dependent characteristic. The independent variables in the F4 generation were kernel diameter, hardness index, protein content, mixing development time and thousand kernel mass. The coefficient of determination (R^2), expressed as a percentage, indicates the proportion of variation that can be explained by the relationship between X and Y.

Table 5.4 Expected ANOVA and expected mean squares in the F4 populations (Rasmusson and Lambert, 1961)

Source	d.f.	Mean squares	Expected Mean Square
Replication	l(r-1)		
Genotype	(g-1)	M_3	$\delta^2_e+r\delta^2_{gl}+rl\delta^2_g$
Location	(l-1)		
Genotype x location	(g-1)(l-1)	M_2	$\delta^2_e+r\delta^2_{gl}$
Error	l(g-1)(r-1)	M_1	δ^2_e

M - mean square, l - location, r - replication, g - genotype

Table 5.5 Calculations to determine variance components in the F4 populations (Rasmusson and Lambert, 1961)

Variance component	Calculation
Genotype (δ^2_g)	$\frac{M_3 - M_2}{r\ l}$
Genotype x location (δ^2_{gl})	$\frac{M_2 - M_1}{R}$
Plot error (δ^2_e)	M_1
Phenotype (δ^2_p)	$\delta^2_g + (\delta^2_{gl}/l) + (\delta^2_e/rl)$
Heritability (b)	$\frac{\delta^2_g}{\delta^2_p}$

5.3 RESULTS AND DISCUSSION

5.3.1. F2 SDS selected and unselected data

The single plant with the highest SDS percentage in the F2 group where the SDS sedimentation was taken into account (selected) was included in the generation of the following year, while a single plant in the unselected group was included randomly, not taking the known SDS sedimentation and SDS percentage values into account (unselected) (Figure 5.2). The term selected and unselected therefore do not indicate whether single plant selection was performed in the group or not, but whether SDS sedimentation was taken into account or not in the selection.

The differences between the SDS percentages of selected and unselected plants varied from small (1.4%) to large (22.16%) and were both negative and positive. However, in most (five of the seven) cases, a higher SDS sedimentation was selected, indicating that by selecting on the basis of SDS sedimentation, compared to selecting only on agronomical adaptation the chance of selecting a plant with a higher SDS sedimentation in the population is higher (Table 5.4).

In populations 3 to 6 the selected group where SDS sedimentation was taken into account had a higher SDS percentage than the unselected group where SDS sedimentation was not taken into account in the F2 generation. The SDS sedimentation of the selected group remained higher than the unselected group in the F4 generation.

In population 1 the selected group where SDS sedimentation was taken into account had a lower SDS percentage than the unselected group where SDS sedimentation was not taken into account in the F2 generation and the SDS sedimentation of the selected group remained lower than the unselected group in the F4 generation. The difference between selected where SDS sedimentation was taken into account and unselected groups where SDS sedimentation was not taken into account for these populations remained constant from the F2 to the F4 generation. This is in accordance with Sunderman *et al*. (1965), who reported medium to high SDS sedimentation heritability (44-64%) in early generation material and Fischer *et al*. (1989) reporting significant positive correlations between SDS sedimentation in the F3 and F7 generation, with a heritability of 44%. SDS sedimentation of a population can therefore be enhanced if selection for SDS is applied in the early generations of the breeding program.

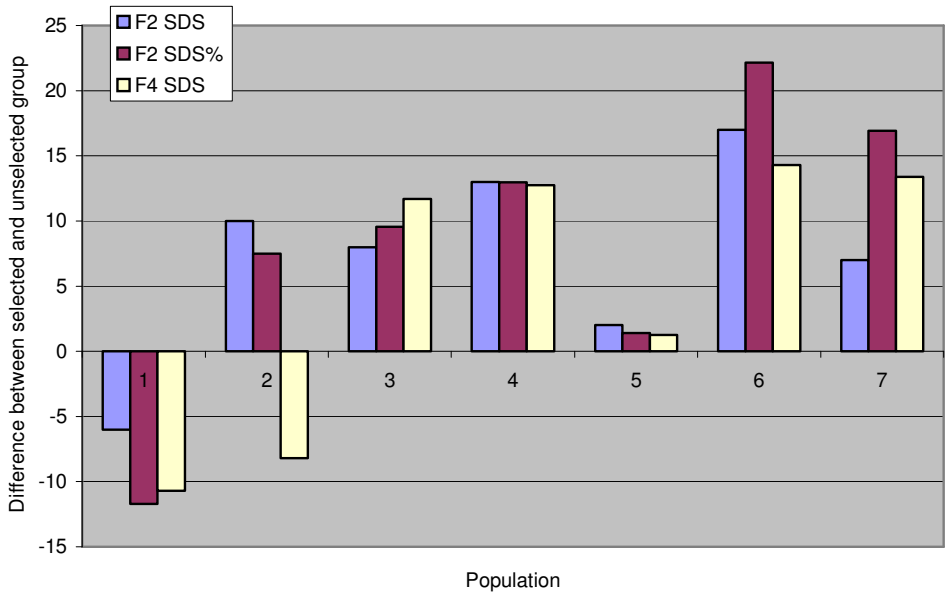


Figure 5.2 Differences between groups where the SDS sedimentation was taken into account (selected) and the group where SDS sedimentation was not taken in account (unselected)

The magnitude of the differences between the groups (selected and unselected) remained consistent from the F2 to the F4 generation for populations 1,3,4,5 and 6. The large within group differences in the F2 generation of populations 1, 3, 4 and 6 remained large in the F4 generation. The small within group differences between selected and unselected groups of population 5 in the F2 generation remained small in the F4 generation.

The differences between selected and unselected groups from the F2 to the F4 generation were not consistent in populations 2 and 7. In the F2 generation of population 2 and 7, the selected group had a higher SDS sedimentation than the unselected group, but not in the F4 generation. This is in accordance with Briggs and Shebeski (1971) reporting that the predictive ability for SDS sedimentation value was inconsistent for different population years. There were significant differences between and within groups in the F4 generation, indicating that SDS sedimentation effectively discriminated between closely related genotypes in the F4 generation and that early generation selection for SDS is very effective. Early generation selection for SDS sedimentation would result in a decrease in the amount of material that needs to be evaluated in the later generations of a breeding program.

The ranking of SDS sedimentation for populations 4 and 6 differed in the F2 and F4 generations and F2 SDS percentage ranked population 6 differently in the F2 and F4

generation than the SDS sedimentation volume Table 5.6). The SDS percentage therefore did not seem superior to SDS sedimentation in ranking genotypes in the F2 to give an indication of the ranking in the F4.

Table 5.6 Values of F2 single plant selections in the F2 and resulting F4 generations

Population	2005 F2 SDS sedimentation	Within group difference	2005 F2 SDS %	Within group difference	2007 F4 SDS sedimentation	Within group difference
	Selected group					
1(2)	57(7)	-6	67.06(7)	-11.69	55.20m (7)	-10.7
2(2)	68(4)	10	80.00(6)	7.5	56.53l (6)	-8.2
3(1)	62(5)	8	95.08(3)	9.57	73.60d (3)	11.7
4(1)	60(6)	13	98.26(1)	12.97	74.87c (2)	12.74
5(5)	70(3)	2	82.35(5)	1.4	72.60e (4)	1.27
6(1)	76(1)	17	95.00(4)	22.16	81.53b (1)	14.3
7(1)	72(2)	7	96.09(2)	16.92	70.53f (5)	13.4
	Unselected group					
1	63(3)		78.75(5)		65.934h	
2	58(5)		72.50(7)		64.735i	
3	54(6)		85.57(2)		62.536j	
4	47(7)		85.29(3)		62.137k	
5	68(1)		80.95(4)		71.332f	
6	59(4)		72.84(6)		67.203g	
7	65(2)		113.01(1)		83.931a	

Group in population column is given in parenthesis. In the rest of the table rank is given in parenthesis, and values followed by different letters are significantly different at P≤ 0.05

5.3.2 F4 quality data

5.3.2.1 SDS sedimentation

The analysis of variance is presented in Table 5.7a. The genotype main effect was highly significant, suggesting there were consistent differences in performance amongst the genotypes, which is in accordance with reports by Fischer *et al.* (1989).

The location main effect and genotype x location interaction were highly significant, indicating there was an interaction between genotype and the environment and that location had an influence on the performance of the genotypes. The significant influence of the environment on SDS sedimentation is in fact confounded by the large effect of protein on SDS sedimentation (Carter *et al*., 1999; De Villiers and Laubscher, 1995; Van Lill *et al*., 1995; Lorenzo and Kronstad, 1987).

The heritability of SDS sedimentation (b value) was estimated to be 94%. This is also an indication of the repeatability of the genotype performance, thus the chance of repeating the SDS sedimentation performance is very high. The choice of locations and the fact that the trial was not repeated over years may serve as an explanation for the exceptional high value. All three of the locations were areas with optimum environmental conditions for wheat production and therefore very much alike.

The estimated variance components are presented in Table 5.8, calculated with the formulas presented in Tables 5.4 and 5.5. The genotypic component contributed 85.96% of the total variation in SDS sedimentation. The large genotype contribution found in this study is in accordance with the findings of Van Lill *et al*, (1995), Carter *et al*. (1999) and Kadar and Moldovan (2003), stating that SDS sedimentation is primarily genetically determined.

The genotype x location component contributed only 12.87% of the total variation in SDS sedimentation, suggesting that testing of the genotypes at different locations may not give a more reliable evaluation of genotype performance and is therefore unnecessary. This is in accordance with the findings in Chapter 3 in advanced generations. This is also in accordance with the finding of Van Lill *et a*l. (1995), where the genotype to genotype x environment variance ratios were 4.4 – in this study the genotype/(genotype x environment) variance ratio was 6.67, underlining the fact that SDS sedimentation is primarily genetically determined.

An ANOVA was done to determine the effect of selection on measured characteristics (Table 5.7b). In this ANOVA, location was treated as a fixed effect and genotype and selected vs.

unselected treatment as random effects. The following discussion will concentrate on the random effects.

The selected vs. unselected treatment main component was highly significant (P≤0.01). The treatment x location interaction was highly significant, indicating that the selected vs. unselected groups reacted differently at different locations. The mean SDS sedimentation values and rank of the genotypes are presented in Table 5.9.

Groups were identified on the basis of how the F4 generation reacted on SDS sedimentation percentage in the F4 generation (Figure 5.2). Three groups could be identified and are indicated within brackets in Table 5.6 after the population number. Group 1 had the low SDS sedimentation types, with the selected group where SDS sedimentation was taken in consideration in the F2 generation having significantly higher values than the unselected group where SDS sedimentation was not taken into consideration in the F2 generation. The genotypes in Group 2 had high SDS sedimentation values, with large significant differences between the selected and unselected groups. The genotypes in Group 3 had high SDS sedimentation values, but the difference between the selected and unselected populations was small. Group 1 included populations 3, 4, 6 and 7. Group 2 included population 1 and 2. Group 3 included population 5.

The result of F2 SDS sedimentation selection on the F4 sedimentation is presented in the within group difference in Table 5.6. There was a significant difference between all the populations, except for unselected population 5 and selected population 7, suggesting SDS sedimentation successfully discriminated between populations. There were significant differences between the selected and unselected groups of all the populations, indicating that SDS sedimentation successfully discriminated between populations and early generation selection for SDS sedimentation is effective.

5.3.2.2 Protein content

Both the genotype and genotype x location components were highly significant (Table 5.7a). The heritability, also an indication of the repeatability of protein content, was 69%. The choice of locations and the fact that the trial was not repeated over years may serve as an explanation for the exceptionally high value. All three of the locations are areas with optimum environmental conditions for wheat production and therefore very much alike.

The estimated variance components are presented in Table 5.8, calculated with the formulas presented in Tables 5.4 and 5.5. The genotype component contributed 22.75%, 63.21% less than the genotype component of the total variation in SDS sedimentation. It can be

concluded that environment plays a large role in the variation detected in protein content and that SDS sedimentation is more consistent than protein content over locations. Since the genotype effect on SDS sedimentation was higher than the genotype main effect on protein content, it appears to be of more value as a selection criterion, as it appears to be subjected less to environmental factors.

It is well known that protein content is strongly influenced by the environment (Kadar and Moldovan, 2003). In the reports by Van Lill *et al*. (1995) the genotype x location component was far greater than the genotype component. In literature it was reported that protein is more influenced by environmental conditions than by genotype and hence is of lesser importance on its own in wheat breeding (Greenaway *et al*., 1966; Van Lill *et al*., 1995). Nel *et al*. (1998) reported that the environment contribution to variation in protein content was by far the largest, with significant genotype x environment interaction. The large location component in protein content indicates that trials would have to be conducted over locations if effective evaluation of the true protein content performance of the genotypes is the aim. In contrast to this, Graybosch *et al*. (1996) reported that protein quality, as measured by the SDS sedimentation, is more susceptible to environmental modification than flour protein content.

Genotype x environment interactions could reduce effectiveness of early generation selection for quality characteristics under certain conditions (Lebsock *et al*., 1964), especially if the trait is strongly influenced by protein content. The location x selected vs. unselected interaction was insignificant (Table 5.7b), indicating that selection of SDS sedimentation in the F2 generation did not influence the protein content in the F4 generation and that the different groups reacted the same at different locations. The mean flour protein content values and rank of the genotypes are presented in Table 5.10. It is clear that in the majority of genotypes there were no significant differences between and within populations (selected and unselected groups).

5.3.2.3 Mixing development time

The analysis of variance of mixing development time is given in Table 5.7a. The genotype effect was highly significant, suggesting that there are consistent differences in performance amongst the genotypes. The heritability of mixing development time was 91%, which was similar to the heritability of SDS sedimentation. The choice of location and the fact that the trial was not repeated over years may serve as an explanation for the exceptionally high value.

The estimated variance components are presented in Table 5.8, calculated with the formulas presented in Table 5.4 and 5.5. The genotype x location interaction was highly significant, suggesting that the genotypes reacted differently in different locations with regard to mixing development time. However, the genotype component (83.26%) contributed 73.33% more than the genotype x location component (9.93%) to variation, which is in accordance with reports by Eagles *et al*. (2002) for dough development time. The genotype variance component was eight times greater than the genotype x location component, which is in accordance with reports by Van Lill *et al.* (1995) that the genotype variance component is seven times higher than the genotype x location component.

The genotype component contribution to the total variation for SDS sedimentation and mixing development time is very high and both traits are of equal value as a selection criterion. This is in contrast with reports by Van Lill *et al.* (1995), reporting that SDS sedimentation is of lesser value than mixing development time as a selection criterion.

The selected vs. unselected groups component was highly significant ($P \leq 0.01$), indicating that SDS sedimentation selection in the F2 had a significant influence on the mixing development time of the selected vs. unselected groups in the F4 generation (Table 5.7b). The location x selected vs. unselected interaction was insignificant, indicating the differences in selected vs. unselected groups were consistent across locations.

Mean values and rank of the mixing development time of the genotypes are presented in Table 5.11. Populations 3, 4 and 7 had significantly higher mixing development times than their respective unselected populations, suggesting that selection of higher SDS sedimentation in the F2 generation of these populations may result in stronger dough in the F4 generation in some of the crosses. This is in accordance with other reports where a high positive correlation between sedimentation tests and dough strength parameters was reported (Zeleny, 1947; Zeleny *et al*., 1960; Zeleny 1962; Dewey, 1963; Lebsock *et al*., 1964; Fowler and de la Roche; 1975a; Blackman and Gill, 1980; Dexter *et al*., 1980; Quick and Donnelly, 1980; Lorenzo and Kronstad, 1987).

Selected groups of populations 1, 5 and 6 did not have significantly different mixing development times than their respective unselected populations. It may be due to the influence of flour protein content, because the selected groups of populations 1 and 5 also did not have significant protein content differences to their respective unselected groups. The lower mixing development time of the selected population 2 than the unselected population 2 cannot be explained.

5.3.2.4 Hardness index

The analysis of variance is presented in Table 5.7a. The genotype main effect was highly significant, indicating there were consistent differences in performance amongst the genotypes. The genotype x location interaction was highly significant, indicating that the specific location has an influence on hardness index. However, the genotype component contributed 70.74%, 47.51% more than the genotype x location component (23.23%) of the total variation in hardness index, suggesting that evaluation of hardness index over locations is not essential. The heritability of hardness index was 85%. This serves as an indication of the repeatability of the genotype performance, thus the chance of repeating the hardness index performance of the genotypes is 85%.

The selected vs. unselected main component and location x selected vs. unselected influence were insignificant, indicating that selection of F2 SDS sedimentation volume did not significantly influence the hardness index of the F4 generation and the different groups reacted similarly over locations (Table 5.7).

5.3.2.5 Thousand kernel mass

The analysis of variance is presented in Table 5. 7. The estimated variance components are presented in Table 5.8, calculated with the formulas presented in Table 5.4 and 5.5. The genotype main effect was significant. The genotype component contributed only 1.17% of the total variation in thousand kernel mass. The influence of genotype x location component was significant and responsible for 24.4% of the variation in thousand kernel mass. It is clear that the location had a greater influence than genotype on thousand-kernel mass, since the genotype to genotype x location interaction variance ratio was 0.05. This is in accordance with Van Lill *et al.* (1995), who reported that the contribution by genotype was 0.3 times smaller than the genotype x location variance component. The heritability, also serving as the repeatability of the trial, was 2%. The chance of repeating the thousand kernel mass performance by the genotypes is thus very low.

The selected vs. unselected main effect and location x selected vs. unselected interaction was insignificant, indicating that selection of SDS sedimentation in the F2 generation did not have an impact on the thousand kernel mass in the F4 generation and that the different groups reacted the same at different locations (Table 5.7b). The mean thousand kernel mass values and rank of the genotypes are presented in Table 5.13. Population 1 had a significantly higher thousand-kernel mass than the rest of the populations, except for the

unselected group of population 7. However, the selected and unselected group of population 1 were not significantly different from each other.

5.3.2.6 Kernel diameter

The analysis of variance is presented in Table 5.7a and estimated variance components presented in Table 5.8. The genotype main effect was significant, but only contributed 8.51% to the total variation in kernel diameter. The location and genotype x location components was insignificant. This indicates that location did not influence the kernel diameter. The residual unexplainable variance was very large. The heritability of kernel diameter was 11%, thus the chance of repeating the kernel diameter performance of the genotypes is very low.

The selected vs. unselected main component and genotype x selected vs. unselected interaction were both insignificant, indicating that selection of SDS sedimentation in the F2 generation did not have a significant effect on the kernel diameter of these groups in the F4 generation (Table 5.7b).

Table 5.7a Analysis of variance for measured characteristics

		SDS sedimentation (ml/5g)		
Component	**df**	**Sum of squares**	**Mean squares**	**Significance**
Total	209	15534.10		
Replication	12	59.63	4.97	**
Genotype	13	13819.56	1063.04	**
Location	2	133.32	66.66	**
Genotype x location	26	1342.41	51.63	**
Residual	156	179.17	1.15	
Grand mean = 68.76			**CV (%) = 1.56**	
R-squared = 0.99			**b value = 0.94**	
		Protein content		
Source	**df**	**Sum of squares**	**Mean squares**	**Significance**
Total	209	428.25		
Replication	12	6.05	0.50	
Genotype	13	52.96	4.07	**
Location	2	249.72	124.86	**
Genotype x location	26	36.86	1.42	**
Residual	156	82.67	0.53	
Grand mean = 12.25			**CV (%) = 5.94**	
R-squared = 0.81			**b value = 0.32**	

Table 5.7a Analysis of variance for measured characteristics (continued)

		Mixing development time		
Source	**df**	**Sum of squares**	**Mean squares**	**Significance**
Total	209	131.90		
Replication	12	0.17	0.015	
Genotype	13	69.83	5.371	**
Location	2	50.19	25.10	**
Genotype x location	26	6.224	0.239	**
Residual	156	5.486	0.035	
Grand mean = 3.153			**CV (%) = 5.95**	
R-squared = 0.96			**b value = 0.91**	
		Hardness index		
Source	**df**	**Sum of squares**	**Mean squares**	**Significance**
Total	209	34963.43		
Replication	12	130.00	10.83	
Genotype	13	24654.34	1896.49	**
Location	2	3151.34	1575.67	**
Genotype x location	26	5146.205	197.931	**
Residual	156	1881.538	12.061	
Grand mean = 51.19			**CV (%) = 6.78**	
R-squared = 0.95			**b value = 0.85**	

Table 5.7a Analysis of variance for characteristics

		Thousand kernel mass (g)		
Source	**df**	**Sum of squares**	**Mean squares**	**Significance**
Total	209	2736.48		
Replication	12	95.369	7.95	
Genotype	13	334.427	25.73	**
Location	2	83.276	41.64	*
Genotype x location	26	618.368	23.783	**
Residual	156	1605.038	10.289	
Grand mean = 39.35			**CV (%) = 8.15**	
R-squared = 0.41			**b value = 0.02**	
		Kernel diameter (mm)		
Source	**df**	**Sum of squares**	**Mean squares**	**Significance**
Total	209	8.06		
Replication	12	0.17	0.01	
Genotype	13	1.19	0.01	**
Location	2	0.03	0.092	
Genotype x location	26	1.27	0.049	
Residual	156	5.40	0.035	
Grand mean = 2.93			**CV (%) = 6.34**	
R-squared = 0.33			**b value = 0.12**	

Table 5.7b Analysis of variance for characteristics for selected and unselected groups

		SDS sedimentation (ml/5g)		
Source	**df**	**Sum of squares**	**Mean squares**	**Significance**
Total	209	15534.10		
Replication	4	52.76	13.19	**
Genotype	13	13819.56	1063.04	**
Selected vs. unselected group	1	53.51	53.51	**
Location	2	133.32	66.66	**
Genotype x location	26	1342.41	51.63	**
Treatment x location	2	32.47	16.23	**
Residual	122	186.04	1.53	
Grand mean = 68.76			**CV (%) = 1.80**	
R-squared = 0.99				
		Protein content (%)		
Source	**df**	**Sum of squares**	**Mean squares**	**Significance**
Total	209	428.25		
Replication	4	2.18	0.55	
Genotype	13	52.96	4.07	**
Selected vs. unselected group	1	0.96	0.96	
Location	2	249.72	124.86	**
Genotype x location	26	36.86	1.42	**
Treatment x location	2	1.48	0.74	
Residual	122	86.54	0.71	
Grand mean = 12.25			**CV (%) = 6.88**	
R-squared = 0.80				

Table 5.7b Analysis of variance for characteristics for selected and unselected groups

		Mixing development time (min)		
Source	**df**	**Sum of squares**	**Mean squares**	**Significance**
Total	209	131.90		
Replication	4	0.05	0.01	
Genotype	13	69.83	5.37	**
Selected vs. unselected group	1	0.53	0.53	**
Location	2	50.19	25.10	**
Genotype x location	26	6.22	0.24	**
Treatment x location	2	0.25	0.13	
Residual	122	5.16	0.05	
Grand mean = 3.15			**CV (%) = 6.80**	
R-squared = 0.96				
		Hardness index (%)		
Source	**df**	**Sum of squares**	**Mean squares**	**Significance**
Total	209	34963.43		
Replication	4	20.08	5.02	
Genotype	13	24654.34	1896.49	**
Selected vs. unselected group	1	38.55	38.55	
Location	2	3151.34	1575.67	**
Genotype x location	26	5146.21	197.93	**
Treatment x location	2	6.16	3.08	
Residual	122	1991.47	16.32	
Grand mean = 51.19			**CV (%) = 7.89**	
R-squared = 0.94				

Table 5.7b Analysis of variance for characteristics for selected and unselected groups

		Thousand kernel mass (g)		
Source	**df**	**Sum of squares**	**Mean squares**	**Significance**
Total	209	2736.48		
Replication	4	20.21	5.05	
Genotype	13	334.43	25.73	*
Selected vs. unselected group	1	0.77	0.77	
Location	2	83.28	41.64	
Genotype x location	26	618.37	23.78	*
Treatment x location	2	44.99	22.50	
Residual	122	1680.19	13.77	
Grand mean = 39.35			**CV (%) = 9.43**	
R-squared = 0.39				
		Kernel diameter (mm)		
Source	**df**	**Sum of squares**	**Mean squares**	**Significance**
Total	209	8.06		
Replication	4	0.05	0.01	
Genotype	13	1.19	0.09	*
Selected vs. unselected group	1	0.09	0.01	
Location	2	0.03	0.01	
Genotype x location	26	1.27	0.05	
Treatment x location	2	0.22	0.11	
Residual	122	5.53	0.05	
Grand mean = 2.93			**CV (%) = 7.25**	
R-squared = 0.31				

Table 5.8 Estimated variance components and their relative contribution to total variation in the different parameters

	Percentage of total variance					
Variance component	**SDS sedimentation**	**Protein content**	**Mixing development time**	**Hardness index**	**Thousand kernel mass**	**Kernel diameter**
Genotype (δ^2_g)	85.96	22.75	83.26	70.74	1.17	8.51
Genotype x location (δ^2_{gl})	12.87	22.79	9.93	23.23	24.40	8.31
Plot error (δ^2_e)	1.17	54.46	6.81	6.03	74.43	83.17
Total	100	100	100	100	100	100

Table 5.9 Mean values and ranking of SDS sedimentation in the F4 population

	SDS sedimentation		
Population	**Mean value**	**CV**	**Rank**
Population 1 Selected	55.20m (-10.7)	3.2	14(7)
Population 2 Selected	56.53l (-8.2)	2.7	13(6)
Population 3 Selected	73.60d (11.7)	4.1	4(3)
Population 4 Selected	74.87c (12.74)	4.1	3(2)
Population 5 Selected	72.60e (1.27)	4.1	5(4)
Population 6 Selected	81.53b (14.3)	3.6	2(1)
Population 7 Selected	70.53f (13.40)	5.3	7(5)
Population 1 Unselected	65.93h	2.3	9(4)
Population 2 Unselected	64.73i	5.9	10(5)
Population 3 Unselected	62.53j	4.4	11(6)
Population 4 Unselected	62.13k	2.7	12(7)
Population 5 Unselected	71.33f	7.7	6(2)
Population 6 Unselected	67.20g	4.0	8(3)
Population 7 Unselected	83.93a	1.7	1(1)
LSD=0.89			

Value in parenthesis for mean values indicates deviation from unselected group. Value in parenthesis for rank indicates within group ranking for selected and unselected groups. Mean values followed by different letters indicate significant differences at P≤0.05.

Table 5.10 Mean values and ranking of protein content in the F4 populations

	Protein content		
Population	**Mean value**	**CV**	**Rank**
Population 1 Selected	11.05c	5.5	14
Population 2 Selected	12.07b	12.2	11
Population 3 Selected	12.48ab	13.9	6
Population 4 Selected	12.66ab	8.0	2
Population 5 Selected	12.55ab	8.9	4
Population 6 Selected	12.88a	8.4	1
Population 7 Selected	12.53ab	7.7	5
Population 1 Unselected	11.26c	5.1	13
Population 2 Unselected	12.47ab	11.0	7
Population 3 Unselected	12.05b	12.0	12
Population 4 Unselected	12.24b	10.4	9
Population 5 Unselected	12.63ab	6.4	3
Population 6 Unselected	12.17b	25.1	10
Population 7 Unselected	12.45ab	7.9	8
LSD=0.61			

Mean values followed by different letters indicate significant differences at P≤0.05

Table 5.11 Mean values and ranking of mixing development time in the F4 populations

	Mixing development time		
Population	**Mean value**	**CV**	**Rank**
Population 1 Selected	2.03g	24.3	14
Population 2 Selected	2.87f	22.0	11
Population 3 Selected	4.03a	13.7	1
Population 4 Selected	3.37d	13.2	6
Population 5 Selected	2.90ef	13.9	10
Population 6 Selected	3.77bc	13.6	3
Population 7 Selected	3.45d	21.2	5
Population 1 Unselected	2.09g	19.3	13
Population 2 Unselected	3.13e	23.2	7
Population 3 Unselected	3.63c	16.5	4
Population 4 Unselected	3.06e	21.4	9
Population 5 Unselected	2.85f	18.2	12
Population 6 Unselected	3.87ab	13.6	2
Population 7 Unselected	3.08e	17.9	8
LSD=0.16			

Means in red indicate that the selected and unselected group within a population is not significantly different from each other. Mean values followed by different letters indicate significant differences at P≤0.05.

Table 5.12 Mean values and ranking of hardness index in the F4 populations

	Hardness index		
Population	**Mean value**	**CV**	**Rank**
Population 1 Selected	24.14g	43.4	14
Population 2 Selected	53.21c	14.4	7
Population 3 Selected	60.50a	12.3	2
Population 4 Selected	54.93bc	13.9	6
Population 5 Selected	60.15a	10.3	4
Population 6 Selected	53.05c	6.2	8
Population 7 Selected	49.35e	11.2	12
Population 1 Unselected	28.85f	38.8	13
Population 2 Unselected	56.63b	13.3	5
Population 3 Unselected	61.30a	12.4	1
Population 4 Unselected	51.85de	15.4	10
Population 5 Unselected	60.48a	11.2	3
Population 6 Unselected	50.01de	5.1	11
Population 7 Unselected	52.55cd	8.5	9
LSD=2.92			

Means in red indicate that the selected and unselected group within a population is not significantly different from each other. Mean values followed by different letters indicate significant differences at P≤0.05.

Table 5.13 Mean values and ranking of thousand kernel mass in the F4 populations

	Thousand kernel mass		
Population	**Mean value**	**CV**	**Rank**
Population 1 Selected	40.84abc	6.8	3
Population 2 Selected	38.74bcde	6.0	10
Population 3 Selected	39.42bcde	9.6	6
Population 4 Selected	38.56bcde	14.2	11
Population 5 Selected	39.02bcde	8.6	9
Population 6 Selected	40.10abcd	8.1	4
Population 7 Selected	38.26cde	4.4	12
Population 1 Unselected	42.18a	6.5	1
Population 2 Unselected	39.05bcde	6.1	7
Population 3 Unselected	39.04bcde	13.0	8
Population 4 Unselected	39.42bcde	9.0	5
Population 5 Unselected	37.38e	8.7	14
Population 6 Unselected	37.79de	11.1	13
Population 7 Unselected	40.98ab	7.8	2
LSD=2.68			

Means in red indicates that the selected and unselected group within a population is not significantly different from each other. Mean values followed by different letters indicate significant differences at P≤0.05.

Table 5.14 Mean values and ranking of kernel diameter in the F4 populations

	Kernel diameter		
Population	**Mean value**	**CV**	**Rank**
Population 1 Selected	3.01a	7.0	4
Population 2 Selected	2.98a	6.8	5
Population 3 Selected	2.90abc	4.3	10
Population 4 Selected	3.01a	5.2	3
Population 5 Selected	2.95ab	4.9	7
Population 6 Selected	2.77c	4.6	14
Population 7 Selected	2.96ab	9.5	6
Population 1 Unselected	3.03a	5.7	2
Population 2 Unselected	3.03a	5.3	1
Population 3 Unselected	2.89abc	5.8	12
Population 4 Unselected	2.90abc	5.7	9
Population 5 Unselected	2.83bc	8.9	13
Population 6 Unselected	2.89abc	7.6	11
Population 7 Unselected	2.91abc	5.5	8
LSD=0.15			

Means in red indicate that the selected and unselected group within a population is not significantly different from each other. Mean values followed by different letters indicate significant differences at P≤0.05.

5.3.3 Canonical variate analysis

Canonical variate analysis (CVA) analysis was done on the averages of the four individual replications of the combined F4 trials to determine if SDS sedimentation is able to discriminate between the different F4 populations. The first two discriminant latent vectors and correlation coefficients of parameters and of cultivars for the quality parameters are presented in Tables 5.15 to 5.17. Latent root 1 was 5.986, which proves this analysis is highly significant (because at least one of the latent roots have to be greater than 1 in order for the analysis to be significant) and clearly differentiated among entries (latent root > 1).

The first two canonical variates (CV1 and CV2) accounted for 90.66% of the variation. The parameters discriminating between the genotypes were mixing development time (r=-0.67), SV (r=-0.601) and SDS sedimentation (r=-0.858), as these correlated the strongest with the CV scores. Mixing development time strongly negatively correlated with the CVA1 score, contrasting populations horizontally. SDS sedimentation and SV strongly negatively correlated with the CV2 score, contrasting the populations vertically.

The canonical variate means for each line (scores for CVA1 and CVA2) were plotted in Figure 5.3. The figure is an indication of how the different populations group for the different parameters that differentiate them effectively over locations. The separation between the genotypes is clear on both the CV1 and CV 2 axes, since it accounted for such a large amount of the variation. Genotypes further apart are dissimilar with regard to the variates that discriminate between them.

Population 1 clearly contrasts from the rest of the populations, mainly because of mixing development time differences. Population 1 had a short mixing development time and a low SDS sedimentation. Populations 6 and 7 had high SDS sedimentation and long mixing development times. Populations 2, 3 and 4 had low SDS sedimentation, but long mixing development times. The populations with the longest mixing development time were populations 5 and 6. The populations with the highest SDS sedimentation were 4, 5, 6 and 7. The populations associating with the highest SV were populations 2 and 3. The populations with the highest protein content were populations 2 and 3.

In Figure, 5.4 a 95% confidence circle indicates significant differences between populations. The radius of the circle is given by the square root of the 95% point of the chi-square variable. Populations 4, 5 and 6 were grouped together, indicating smaller differences between these than the rest of the populations.

Table 5.15 Latent vectors as an indication of the correlation between the parameters

	X-axis	Y-axis			
Latent roots	**CV1**	**CV2**	**CV3**	**CV4**	**CV5**
Variation explained (%)	0.00	0.001	0.005	-0.002	0.005
SDS volume (mL/5g)	0.387	-0.610	-0.178	-0.798	-0.091
Flour protein content (%)	-2.346	2.866	-0.181	4.807	0.056
Flour yield (%)	-1.358	-0.389	1.993	-0.532	-0.320
SV	-4.759	5.662	1.907	10.522	1.260
Mixing development time (minutes)	0.017	0.043	0.017	0.048	0.015
Yield (ton/ha)	-0.060	1.195	-0.669	2.553	-3.569
SV	-0.149	0.043	-0.055	0.001	0.012

CVA1 - first canonical variate, CVA2 - Second canonical variate

Table 5.16 Correlation matrix between the variables and CVA scores

	SDS volume	Falling number	Kernel diameter	Flour protein	Mixing development	Grain yield	SV
CVA1	-0.372	-0.17	0.22	-0.38	-0.67	0.25	-0.10
CVA2	-0.858	0.08	0.27	-0.22	-0.25	-0.01	-0.60
CVA3	-0.152	0.03	-0.05	-0.14	0.47	0.19	-0.00

*** Coefficient significant at P≤0.05**

Table 5.17 Latent vectors as an indication of the correlation between the genotypes

	X-axis	Y-axis			
	CV1	**CV2**	**CV3**	**CV4**	**CV5**
Population 1	5.5751	0.1795	0.3600	0.0079	0.0929
Population 2	-0.3888	1.5740	-0.2567	-0.1384	-0.1619
Population 3	-2.6874	0.6228	1.0903	0.2246	0.1983
Population 4	-0.4942	0.2078	-0.1965	0.1089	-0.1998
Population 5	-0.9241	-0.1390	-1.4586	-0.1164	0.2198
Population 6	-0.9683	-1.1505	0.6656	-0.5167	-0.0464
Population 7	-0.1123	-1.2946	-0.2041	0.4301	-0.1030

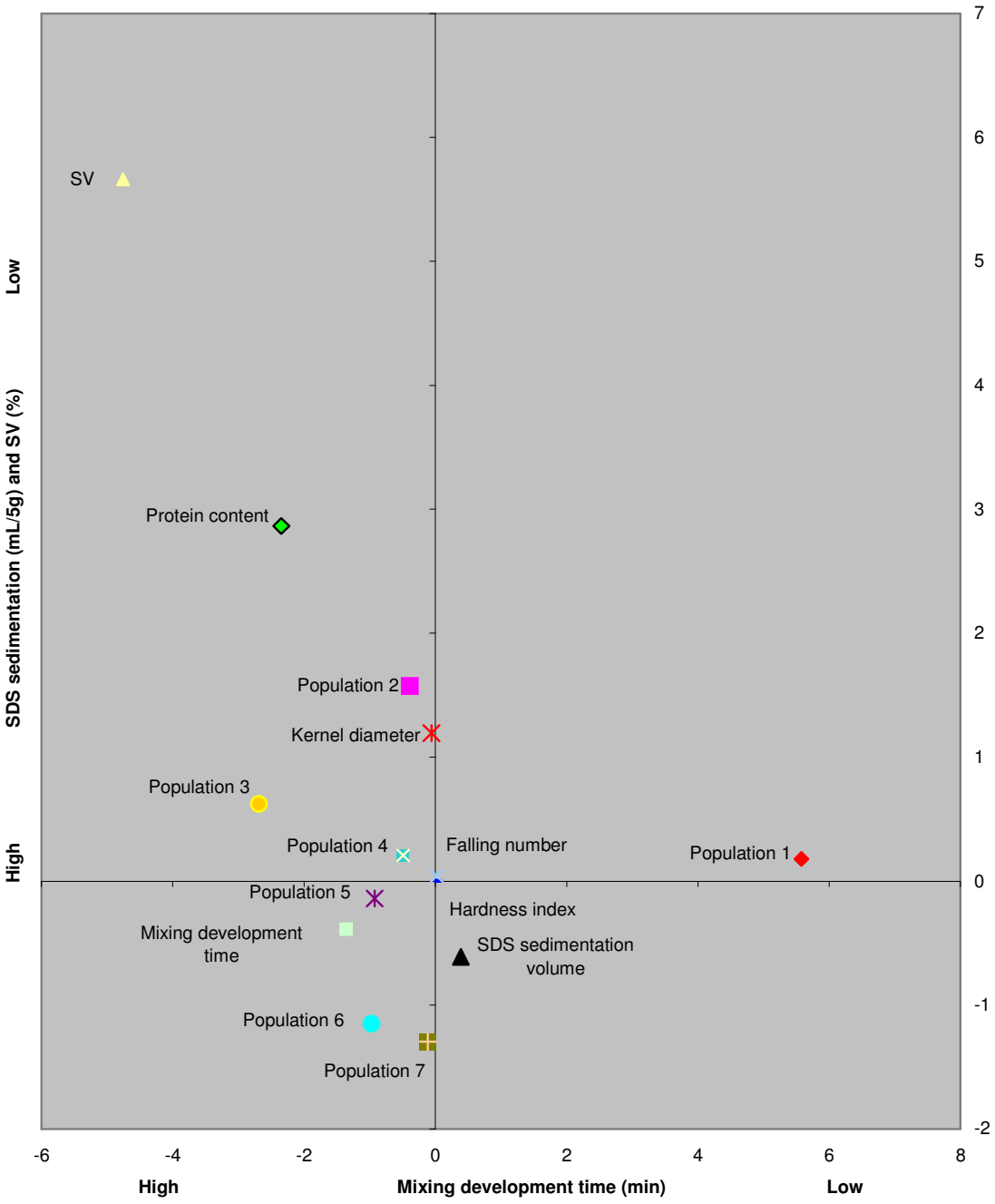


Figure 5.3 Grouping of parameters at three locations in the Eastern Free State

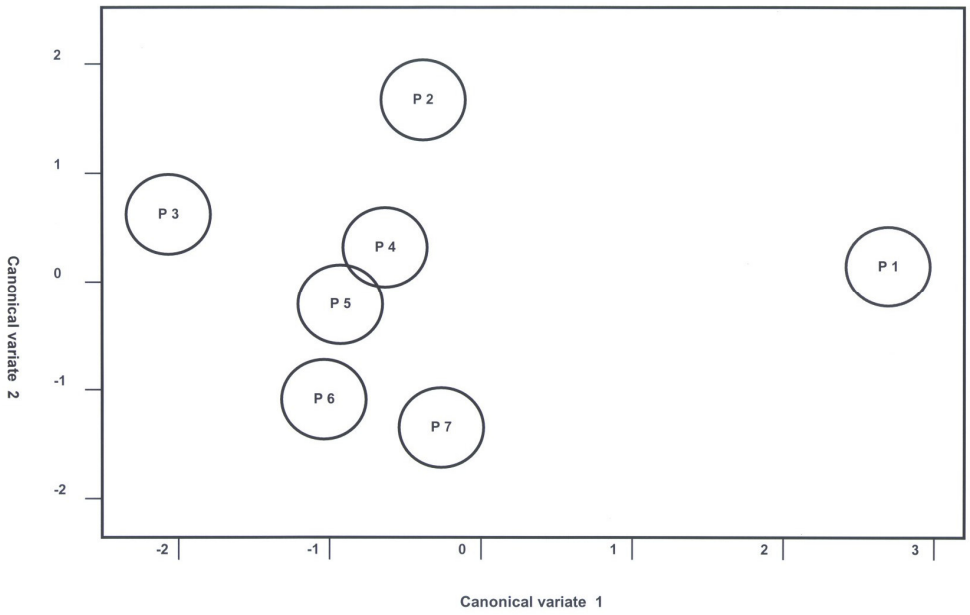


Figure 5.4 Canonical variate analyses of the genotypes

5.3.4 Additive main effects and multiplicative Interaction

The additive main effects and multiplicative interaction (AMMI) is presented in Figure 5.5. Only the first IPCA axis is presented in the analysis, since it removes most of the noise in the data. The ANOVA is given in Table 5.18. Genotype, location and treatment (selected vs. unselected) were highly significant. The genotype x environment interaction was insignificant, indicating the different populations reacted similarly in the different locations.

By selecting populations with a higher performance in combination with high stability, a reliable increase in SDS sedimentation can be expected. Populations 6 and 7 had high SDS sedimentation values (performance) and high stability. Population 5 had high SDS sedimentation, but low stability. Populations 1 and 2 had low SDS sedimentation, but moderate stability. Populations 3 and 4 had moderate SDS sedimentation and low stability.

The further away from zero the IPC score for the location, the more interaction the environment has with the genotypes at that location and the more difficult it will be to select genotypes adapted to the specific location. By selecting environments that successfully discriminate between genotypes and environments that produce consistent data, the breeding program's effectiveness can be increased. Meets and Clarens were able to differentiate effectively amongst the populations, whereas Bethlehem had lower discrimination ability, but were more stable (Figure 5.5).

Table 5.18 AMMI analysis of variance for SDS sedimentation

Component	df	Sum of squares	Mean squares	Significance
Total	209	15534	74.3	
Treatment	20	8419	421.0	**
Location	2	133	66.7	**
Replication	27	165	6.1	
Genotype	6	7417	1236.2	**
Genotype x environment	12	869	72.4	
IPCA	7	625	89.2	*
Residual	5	244	48.8	
Error	162		42.9	

Table 5.19 Principal component analysis (IPCA 1) scores plotted against SDS sedimentation for populations

	IPCA 1 Score	Mean
Population 1	-0.55	60.57
Population 2	0.56	60.63
Population 3	-1.20	68.07
Population 4	-0.76	68.50
Population 5	2.04	71.97
Population 6	0.70	74.37
Population 7	-0.79	77.23

Table 5.20 Principal component analysis (IPCA 1) scores plotted against SDS sedimentation for locations

	IPCA 1 Score	Mean
Bethlehem	0.05	68.13
Meets	1.96	69.89
Clarens	-2.01	68.27

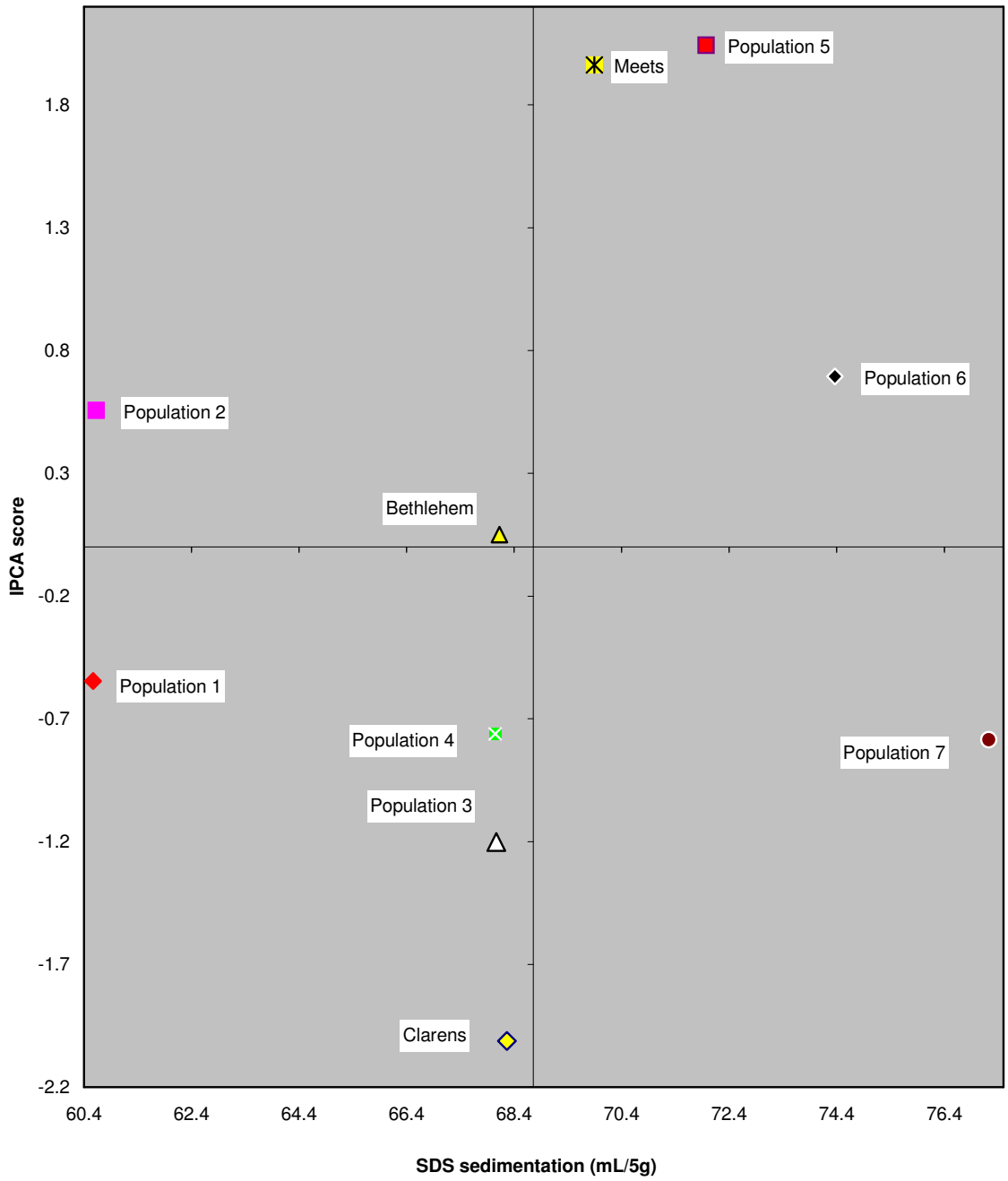


Figure 5.5 Grouping of populations at three locations in the Eastern Free State

5.3.5 Correlations

SDS sedimentation and protein content

In accordance with the results in the advanced material (Chapter 4, Table 4.24), the relationship between SDS sedimentation and flour protein content was highly significant (r=0.28) (Table 5.21), which is in accordance with reports in the literature (Moonen *et al*., 1982; Dick and Quick, 1983; Lorenzo and Kronstad, 1987; Fischer *et al*., 1989; De Villiers and Laubscher, 1995; Carter *et al*., 1999; Cubadda *et al*., 2007). However, Preston *et al*., (1982) stated that SDS sedimentation measures not only differences in protein content, but rather differences in protein quality, which might explain the observed low correlation between SDS sedimentation and protein content (Table 5.21). Furthermore, Maghirang *et al*. (2006) stated that SDS sedimentation is not only affected solely by protein content but possibly also some non-protein components such as pentosans, damaged starch, tailings and others. To support this, no significant or very low correlation between SDS sedimentation and protein content was reported (Preston *et al*., 1982; Dhaliwal *et al*., 1987; Ruix and Carrillo, 1995).

Table 5.21 Relationship between SDS sedimentation and protein content as reported in literature

Correlation coefficient (r)	Reference
0.38** (F2 material)	Sunderman *et al*. (1965)
0.64** (F3 material)	Sunderman *et al*. (1965)
0.48	Moonen *et al*. (1982)
0.63*-0.74* (different sets of germplasm)	Preston *et al.* (1982)
0.69** (5g of flour)	Krattiger and Law (1991)
0.64** (1g of flour)	Krattiger and Law (1991)
0.67 (150kg $N.ha^{-1}$)	De Villiers and Laubscher (1995)
0.58 (150kg $N.ha^{-1}$)	De Villiers and Laubscher (1995)
0.78	Finney and Bains (1999)
0.75 (hard red winter wheat)	Maghriang *et al*. (2006)
0.50 (hard red spring wheat)	Maghirang *et al*. (2006)

0.56*	Colombo *et al*. (2008)	

SDS sedimentation and mixing development time

The relationship between SDS sedimentation and dough strength was highly significant (r=0.35), which was also evident in the advanced material (Chapter 4, Table 4.24). The strong relationship between these two parameters has previously been reported in common wheat (Zeleny *et al*., 1960; Moonen *et al*., 1982; Preston *et al*, 1982; Dhaliwal, 1987; Sapirstein and Suchy, 1999) and durum wheat (Dexter *et al*., 1980; Quick and Donnelly, 1980). It can therefore be possible that selection of high SDS sedimentation may result in excessively strong dough.

SDS sedimentation and hardness index

There was a highly significant relationship between SDS sedimentation volume and hardness index. The reason for this seems unclear, but it may be possible that the gene coding for gene hardness might have an influence on the gluten quality of wheat. The direct influence of the hardness locus on SDS sedimentation volumes have not been investigated and needs investigation in the future.

SDS sedimentation and thousand kernel mass

There were no significant relationship between SDS volume and thousand kernel mass.

SDS sedimentation and kernel diameter

A highly significant negative relationship between SDS sedimentation volume and kernel diameter was observed (r=-0.2). This might be related to protein content since smaller kernels are expected to have higher protein content, which results in higher SDS sedimentation volumes.

SDS sedimentation and falling number

No significant relationship between SDS sedimentation volume and falling number exists. This is in accordance with the finding in the advanced material (Chapter 4, Table 4.24). The falling number test was terminated at 410s. This may have influenced the relationship between SDS sedimentation volume and falling number.

Table 5.21 Combined correlation matrix of the F4 populations from three locations in the Eastern Free State

	SDS	FN	FPC	MDT	TKM	DAM
Falling number (FN)	0.06					
Flour protein content (FPC)	0.28 **	0.37 **				
Mixing development time (MDT)	0.35 **	0.18 **	0.57 **			
Thousand kernel mass (TKM)	0.03	0.27	-0.04	-0.07		
Kernel diameter (DAM)	-0.20 **	-0.03	-0.14	-0.18	0.00	
Hardness index (HI)	0.27 **	0.12	0.04	0.24 **	-0.26 **	-0.19 **

**** P≤0.01, * P≤0.05**

5.3.6 Multiple stepwise regression

Multiple stepwise regression was used to determine the character that contributes the most to the variation in SDS sedimentation, the dependent characteristic. The independent variables in the F4 generation were protein content, mixing development time, falling number, thousand kernel mass, kernel diameter and hardness index. The coefficient of determination (R^2), expressed as a percentage, indicates the proportion of variation that can be explained by the relationship between X and Y. Mixing development time and hardness index contributed the most to SDS sedimentation (Table 5.22). It was possible to explain 8.11% and 7.95% of the variation in SDS sedimentation due to mixing development time and hardness index respectively. The other characteristics made only minor contributions and were of insignificant value in predicting variation in SDS sedimentation.

Table 5.22 Adjusted R for all the traits in the model, explaining the variation in SDS sedimentation in the F4 material (P≤0.05) at the three localities

Locality	Action	Variable	R^2	Mean square error	Model p-level
0	Unchanged		0.00	8.62	0.00
1	Added	MDT	0.12	8.11	0.00
2	Added	HI	0.16	7.95	0.06
3	Unchanged		0.16	7.95	0.06

5.4 CONCLUSIONS

It is clear from this study that SDS sedimentation of a population can be enhanced if selection for SDS is applied in the early generations of the breeding program. By selecting on the basis of SDS percentage of single plants in the F2 generation compared to selecting only on agronomical adaptability, the chance of selecting a plant with a higher SDS sedimentation in the population is higher. The difference in SDS sedimentation between selected and unselected groups for most populations remained constant from the F2 to the F4 generation. Effective SDS selection in the early generations of a breeding programme is therefore possible to improve bread baking quality. Early generation SDS sedimentation selection would decrease the amount of material that needs to be evaluated in advanced generations of the program. If the amount of material is effectively reduced in the early generations of a program, the effectiveness of a breeding program will dramatically be improved, both financially and practically.

There were significant differences between all the selected and unselected populations in the F4 as a result of selection in the F2, except for the unselected population 5 and selected population 7, suggesting SDS sedimentation volume successfully discriminated between populations. There were significant differences between the F2 selected and unselected groups of all the populations in the F4, indicating SDS sedimentation volume successfully discriminated between populations. The SDS percentage did not seem superior to SDS sedimentation in ranking genotypes in the F2 as a preliminary indication of the ranking in the F4.

The heritability of SDS sedimentation volume was estimated to be 94%. This is also an indication of the repeatability of the genotype performance (b value) thus the chance of repeating the SDS sedimentation volume is very high. The choice of locations and the fact that the trials were not repeated over years may serve as an explanation for the exceptional high value. All three locations were areas with optimum environmental conditions for wheat production and therefore they were very much alike, which may also contribute to an exceptionally high repeatability value.

Genotype had the largest influence on SDS sedimentation volume, which is in accordance with the findings of Van Lill *et al*. (1995); Carter *et al*. (1999) and Kadar and Moldovan (2003). Location, population x location interaction and location x selected vs. unselected interaction had a significant influence on the variation in SDS sedimentation volume, but the contribution of these components to the total variation in SDS sedimentation volume was small. SDS

sedimentation volume is therefore mainly influenced by genotype, with small influence from location. This is in accordance with the findings in Chapter 3 where it was concluded that evaluation over locations is less important than evaluation over seasons.

Although selected vs. unselected treatment component contributed only 34% of the total variation in SDS sedimentation volume, the differences between the selected and unselected groups was highly significant ($P \leq 0.01$). Selection of SDS sedimentation within a genotype in the F2 generation therefore has an influence on the SDS sedimentation in the F4 generation and F2 selection of higher SDS sedimentation can result in enhanced SDS sedimentation in later generations.

Single plants within the checks varied considerably. Expressing the single plant SDS sedimentation volume as a percentage of the two nearest neighbouring single checks may therefore have influenced the discrimination ability between genotypes, between single plants within a genotype and the relationship between F2 and F4 SDS sedimentation volumes. It may be more effective to use a bulked row than single plants as a check value in order to eliminate the variability between single plants within the check values. Comparison between the effectiveness of using bulked rows opposed to single plants, as a check value needs further investigation.

The genotype component contribution to the total variation for SDS sedimentation volume and mixing development time was both very high and both traits are of equal value as a selection criterion. This is in contrast with reports by Van Lill *et al*. (1995), reporting that SDS sedimentation volume is of lesser value than mixing development time as a selection criterion, since it was reportedly more influenced by the environment.

The CVA successfully discriminated between five of the seven populations on the basis of SDS sedimentation, SV and mixing development time. The strong relationship between SDS sedimentation and protein content is expected, since it is well known that SDS sedimentation volume is dependent on protein quantity and quality. It is also in accordance with the findings in Chapter 4. The relationship between SDS sedimentation and mixing development time was highly significant, which is in accordance with the findings in Chapter 4. The strong relationship between SDS sedimentation and mixing development time may result in breeding of excessive strong dough if selecting on high SDS sedimentation only. The relationship between SDS sedimentation and hardness index was highly significant. The influence mixing development time and hardness index have on SDS sedimentation was confirmed in the stepwise regression and could therefore be regarded as the most definable independent variance predictors for SDS sedimentation.

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CHAPTER 6
CONCLUSIONS AND RECOMMENDATIONS

There was a significant correlation between baking volume and SDS sedimentation and it was evident that SDS sedimentation and flour protein content could be regarded as the most definable independent predictors for baking volume. Genotypes that ranked the highest for SDS sedimentation also ranked the highest in baking volume. The genotypes that ranked lower in SDS sedimentation volume also ranked lower in loaf volume.

Both SDS sedimentation and mixing development time were highly genotype dependent, indicating that effective selection is indeed possible for both of these traits. It is recommended, however, that preference should be given to SDS sedimentation selection in early generations because of its defining role in the variation of baking volume, which is one of the most important assessments of wheat quality in the South African baking industry.

SDS sedimentation was highly genotype dependent, indicating that there are consistent differences between genotypes over seasons and years and that improvement through selection is possible. SDS sedimentation was less influenced by the locality than by year, with the genotype x locality interaction being smaller than the genotype x year interaction, suggesting that testing of genotypes across years may be more important than testing of genotypes across locations, especially in programs with limited financial resources. The genotype x location x year component of SDS sedimentation volume was relatively small, but highly significant. It can be therefore can concluded that although testing of genotypes across years may be more important, testing of genotypes across localities is also essential for reliable evaluation.

Four to five years is essential for reliable SDS sedimentation selection. This can be done by selecting in the F2 to F5 generations in a breeding program, which will result in a decrease in variation and an improvement in SDS sedimentation stability. This will result in a drastic decrease in the effect of genotype x environment interaction, which will make further selection later in the breeding program for SDS sedimentation selection unnecessary.

The gains in precision obtained by increasing the number of years were substantially more than by increasing the number of locations. In order to increase the precision of SDS sedimentation selection, an increase in the number of years rather than an increase in the

number of locations is recommended. To perform effective SDS sedimentation selection in early generations, testing at one or two locations is adequate. A further increase of locations will result in a very small increase in precision, but would have large practical and financial implications and is therefore not recommended.

It is evident that mixing development time and hardness index could be regarded as the most definable independent variance predictors for SDS sedimentation in the F4 material. The influence and relationship of grain hardness on SDS sedimentation needs further investigation. Grain yield and mixing development time contributed the most to SDS sedimentation in the elite material. Therefore mixing development time and SDS sedimentation are both influenced and related to the same factor, gluten protein composition. There was also a strong relationship between SDS sedimentation volume and dough strength in both early and advanced generations, which was expected, since gluten responsible for the dough strength includes the glutenin, which takes part in the flocculation action of the SDS sedimentation volume process. Care should, however, be taken, that SDS sedimentation volume selection in the early generations of a breeding program does not result in excessive dough strength in subsequent generations. The tendency, however, varies depending on the specific genotype and improvement in SDS sedimentation without affecting dough strength is therefore attainable in certain genotypes. It is recommended that parameters evaluating dough strength accompany SDS sedimentation selection.

The relationship between protein quantity (content) and SDS sedimentation varied. In some cases there was a highly significant relationship between these two traits and in other cases not. Since glutenin makes up part of the flour particles participating in the biochemical process, resulting in flocculation of insoluble constituents, differences in the glutenin quantity and quality might be responsible for the differences

The negative correlation between SDS sedimentation and grain yield was highly significant. This could possibly be attributed to the influence of protein content, because it is well known that a negative association exists between protein content and grain yield, which was also evident in this study. Selection on SDS sedimentation alone could ultimately lead to a yield reduction in subsequent generations and it is therefore recommended that grain yield should be taken into consideration when doing SDS sedimentation selection. Since measuring grain yield is a non-destructive, simple procedure it should not pose a problem to any breeder, whether it is single plant, rows or plots that are being measured.

Effective selection can be made by SDS sedimentation selection of single plants within an early generation population with the aim to improve bread baking quality. SDS sedimentation selection in early generations will therefore drastically decrease the amount of material that needs to be evaluated in the following generations, which will have financial and practical implications for a breeding program.

The discrimination ability of SDS sedimentation in material from a more diverse range of protein content and quality genotypes will be more effective than was the case with this study where the material used was chosen to give a true reflection of the genetic background of a commercial breeding program. Despite this, it was proven that SDS sedimentation is an effective trait to distinguish between genotypes with closely related backgrounds, especially if also taking protein content, mixing development time and hardness index into account. The effectiveness, however, depends on the trial layout, calculation method and selection method used. These aspects need further investigation in South Africa in the dry land wheat breeding program.

By expressing the SDS sedimentation as a percentage of the check on a single plant basis, the breeder assumes that no variation exists between single plants within the check. The performance of individual plants depends strongly on individual conditions, where certain minute factors like seed placement, access to moisture, and rocks in the soil that cannot be controlled, has an influence. The variability within the check may result in the selection of lower SDS sedimentation volume values than the optimum, only because the check values were “accidentally low” and the entry value moderate to high. This might possibly explain the disappointing results in one or two of the populations where only a small advance in selection was made. By taking the mean of a number of plants of the check, such a tendency might be reduced.

The SDS sedimentation test needs to be adjusted and optimised in cases where discrimination between hard genotypes with strong, high-protein characteristics must be done in early generations. Sample weight (5 gram in this study) needs to be reduced and the first reading of swelling time (30 minutes in this study) needs to be reduced to an optimum time. Sufficient SDS stock solution should be available to accommodate strong genotypes with high protein content and thus a higher requirement for sodium dodecyl sulphate in order to provide adequate opportunity for swelling of constituents in all the lines.

CHAPTER 7

SUMMARY

Currently, high numbers of genotypes are unnecessarily being carried from generation to generation in the early generations of wheat breeding programs, only to be discarded in the advanced generations because of not reaching the strict quality standards. If a simple and reliable test could be used as an indication for baking quality in the early generations of the program, a more effective way to do culling of poor quality lines would be possible.

Advanced (elite) and early generation material (F4) were evaluated to determine the influence of genotype and environment on the variation detected in SDS sedimentation. Firstly, nine intermediate hard red wheat elite lines and two intermediate hard red cultivars were evaluated for three consecutive years over eight locations in the Eastern Free State. Secondly, six F4 populations and two hard red cultivars were evaluated for one season at three locations in the Eastern Free State. Single plants in the F2 generation were selected by taking SDS sedimentation into consideration (selected group) and without taking SDS sedimentation into consideration (unselected group) and the influence of selection on the subsequent F4 generations was determined.

The following conclusions were reached in the study:

- There is a significant correlation between baking volume and SDS sedimentation. SDS sedimentation is therefore a reliable predictor of baking volume.
- SDS sedimentation successfully discriminated between the genotypes in both the F4 and elite material. The contribution by genotype was high enough to make effective selection for SDS sedimentation possible. The large genotype x year effect on performance in contrast to the genotype x location effect in the elites, indicated that testing of genotypes across years may be more important than testing of genotypes across locations.
- There were significant differences between the groups where selection was done on SDS sedimentation (selected) and groups where selection was done without taking SDS sedimentation into consideration (unselected groups) in most of the populations. The difference in SDS sedimentation between selected and unselected groups for most populations remained constant from the F2 to the F4 generation. Early generation SDS sedimentation selection would therefore effectively enhance baking quality in later generations. This would decrease the amount of lines that have to be

evaluated in later generations and would have practical and financial advantages for a breeding program.

- In both the elite and F4 material, there was a strong relationship between SDS sedimentation and dough strength, presumably because gluten responsible for the dough strength includes the glutenin, which takes part in the flocculation action of the SDS sedimentation process. SDS sedimentation selection in the early generations of a breeding program can therefore result in excessive dough strength in subsequent generations of certain crosses if a trait evaluating dough strength does not accompany SDS sedimentation selection.
- The relationship between protein quantity (content) and SDS sedimentation varied, possibly due to difference in the glutenin quantity and quality between the genotypes. The negative correlation between SDS sedimentation and grain yield was highly significant. This could possibly be attributed to the influence of protein content, because it is well known that a negative association exists between protein content and grain yield, which was also evident in the study.
- Mixing development time and hardness index (F4 generation) and grain yield and mixing development time (elite material) are the most definable independent variance predictors for SDS sedimentation in the F4 material. Therefore, mixing development time has a large influence on SDS sedimentation or is related to the same aspect, presumably gluten.
- To perform effective SDS sedimentation selection in early generations, testing at one to two locations is adequate. A further increase of locations will result in a very small increase in precision, but would have large practical and financial implications and is therefore not recommended. The gains in precision obtained by increasing the number of years were substantially more than by increasing the number of replications. It is recommended that four to five years is essential for reliable SDS sedimentation selection. This can be done by selecting in the F2 to F5 generations in a breeding program, which will result in a decrease in variation and an improvement in SDS sedimentation stability. This will result in a drastic decrease in the effect of genotype x environmental interaction, which will make further selection later in the breeding program for SDS sedimentation selection unnecessary.

OPSOMMING

`n Groot aantal lyne word onnodig van generasie tot generasie oorgedra in die vroeë generasies van koringteeltprogramme, om later weer uitgeselekteer te word omdat dit te kort skiet aan die streng kwaliteitsvereistes vir vrystelling. `n Eenvoudige, betroubare parameter

wat `n goeie aanduiding van bakkwaliteit gee in die vroeë generasies van die teelprogram, sal effektiewe seleksie vroeg in die program moontlik maak.

Gevorderde (elite) en vroeë-generasie (F4) materiaal is evalueer om te bepaal wat die invloed van omgewing en genotipe op die variasie in SDS sedimentasie is. Eerstens is nege intermediêre harde rooi elite lyne en twee intermediêre harde rooi kultivars geëvalueer vir drie opeenvolgende seisoene by agt lokaliteite in die Oos-Vrystaat. Tweedens is ses F4 populasies en twee harde rooi kultivars geëvalueer vir een seisoen by drie lokaliteite in die Oos-Vrystaat. Die invloed van F2 SDS sedimentasie seleksie op die F4 generasies is bepaal. In die F2 generasie se geselekteerde groep is enkelplante geselekteer met die hoogste SDS sedimentasie volume. In die ongeselekteerde groep is daar bloot op agronomiese voorkoms geselekteer, sonder om die SDS sedimentasie in ag te neem.

Dievolgende gevolgtrekkings kan uit die studie gemaak word:

- Daar is `n betekenisvolle korrelasie tussen bakvolume en SDS sedimentasie. SDS sedimentasie kan dus as `n betroubare aanduiding van bakvolume gebruik word.
- SDS sedimentation het effektief tussen die genotipes in beide die F4 en elite materiaal diskrimineer. Die bydrae deur genotipe was hoog genoeg om effektiewe seleksie vir SDS sedimentasie moontlik te maak. Die groot bydra deur die genotipe x jaar interaksie dui aan dat evaluering van SDS sedimentasie oor jare meer belangrik is as evaluering oor lokaliteite.
- Daar was betekenisvolle verskille tussen groepe waar vir die hoogste SDS sedimentasie geselekteer is (geselekteerde groep) en waar seleksie gedoen is bloot op agronomiese voorkoms, sonder om SDS sedimentasie in ag te neem (ongeselekteerde groep). Die verskil in SDS sedimentasie tussen die geselekteerde en ongeselekteerde groepe het konstant gebly van die F2 na die F4 generasie. Vroeë generasie seleksie vir SDS sedimentasie sal dus effektief selekteer vir hoër bakvolumes in latere generasies. Dit sal die hoeveelheid lyne wat in latere generasie geëvalueer moet word verminder, wat praktiese en finansiële voordele vir `n teelprogram inhou.
- In beide die elite en F4 materiaal was die korrelasie tussen SDS sedimentasie en miksograaf mengtyd hoogs betekenisvol, moontlik omdat glutien glutenien bevat wat verantwoordelik is vir die flokkulering in die SDS sedimentasie proses en glutien ook verantwoordelik is vir deegsterkte. SDS sedimentasie seleksie in die vroeë generasies van `n teelprogram kan lei tot te sterk deeg mengeienskappe as dit nie gekombineer word met ‘n parameter wat deegsterkte ook evalueer nie.

- SDS sedimentasie en proteïeninhoud was hoogs betekenisvol in sommige gevalle, maar nie betekenisvol in ander, moontlik as gevolg van die verskille in die hoeveelheid en kwaliteit glutenien wat variëer het. Daar was `n negatiewe korrelasie tussen SDS sedimentasie en graanopbrengs in die elite materiaal. Dit kan moontlik toegeskryf word aan die invloed van proteïeninhoud, aangesien `n negatiewe korrelasie tussen graanopbrengs en proteïen-inhoud oor die algemeen bestaan, wat ook sigbaar was in die studie.
- Miksograaf mengtyd en hardheid (F4 generasie) en graanopbrengs en miksograafmengtyd (elite generasie) is die eienskappe wat die meeste bydrae tot die variasie in SDS sedimentasie. Dit is dus duidelik dat miksograafmengtyd `n groot invloed op SDS sedimentasie het.
- Een tot twee lokaliteite is voldoende om suksesvol vir SDS sedimentasie te selekteer in vroeë generasies. `n Verdere vermeerdering van die aantal lokaliteite sal slegs tot `n klein toename in proefakkuraatheid lei, maar groot praktiese en finansiële implikasies hê en word daarom nie aanbeveel nie. Die toename in proefakkuraatheid deur die aantal jare te vermeerder is baie groter as die toename in akkuraatheid met die vermeerdering van die aantal lokaliteite.
- Evaluering vir vier tot vyf jaar is nodig vir betroubare evaluering van SDS sedimentasie. Dit kan gedoen word in die F2 tot F5 generasie van `n teelprogram, wat `n vermindering in die variasie en verbetering van stabiliteit vir SDS sedimentasie tot gevolg sal hê. Dit sal ook lei tot `n drastiese verlaging van die effek van genotipe x omgewings interaksie, wat verdere seleksie later in die program onnodig sal maak.

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