MOLECULAR MARKERS FOR AND QUALITY ASSESSMENT OF NEAR ISOGENIC PALMIET AND SST66 WHEAT LINES FOR *PSEUDOCERCOSPORELLA HERPOTRICHOIDES* (EYESPOT) RESISTANCE.

by

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Chapter 1

Introduction

The development of high-yielding varieties of superior quality is the principle objective and challenge of the wheat breeder. However in order to allow the superior quality and high yield potential of the cultivar to be realised and maintained, diseases must be controlled (Johnson and Lupton, 1987).

Until recent times the major determinant of profit was yield, rather than quality. This is now changing with world markets becoming more quality conscious. Plant diseases affecting yield are thus economically very important and finding resistance against it, even more so.

One such a disease is eyespot, caused by the fungus, *Pseudocercosporella herpotricoides* (Fron) Deighton. It is a widespread disease of cereal crops grown in maritime conditions, occurring in the Western Cape region of South Africa. In the case of severe infections, crop losses of up to 50% can occur. The lesions may reduce the 1 000 kernel mass, the number of kernels per head and the tillers per square metre (Scott and Hollins, 1974).

Previously the disease was controlled with fungicides and with biological methods. Recently it has been found that fungicide resistant strains of the pathogen have developed (King and Griffin, 1985). Genetic control is the most preferable means of control. It is cost-effective, environmentally friendly and sustainable.

A few sources of resistance have been identified. The most extensively used resistance gene in breeding programmes is *Pch 1,* derived from *Triticum. ventricosum Ces.* This is a single dominant gene found on chromosome 7D (Cadle *et al.*, 1997).

This single gene is linked to an endopeptidase marker, *Ep-D1b*, and a RFLP marker, Xpsr121 (De La Pena and Murray, 1995).

This gene can be used to control the disease and decrease yield losses. However the gene's effect on baking quality and yield has not been studied.

The aim of this study was to determine whether the presence of the *Pch1* gene has an effect on breadmaking quality and yield in two genetic backgrounds, to find a molecular marker linked to the gene, using the AFLP technique and to look at the protein profiles of the NIL's and their recurrent parents.

Chapter 2

Literature review

The criteria of wheat quality for baking are as varied as its uses (Halverson and Zeleny, 1988). Protein quality and quantity are considered primary factors in measuring the potential of a flour in relation to its end use (Mailhot and Patton, 1988). Wheat proteins contribute to the functionality of flour in the breadmaking process in two distinct ways: the bread flour must have a relatively high protein content, secondly, the protein must have the right quality. Some of the basic quality analysis done on wheat grain and flour before cultivar releases will be discussed in the following paragraphs.

2.1 Milling characteristics

2.1.1 Grain protein

The protein content of wheat grain can vary from 6% to as much as 25%, depending on the growing conditions. Grain protein is a major contributor to the nutritional quality of wheat. In South Africa grain protein of 12% and higher is preferable. The availability of nitrogen is the major determining factor for the protein content of grain (Blackman and Payne, 1987).

There is a strong negative relationship between the grain protein percentage and the grain yield. The rare varieties, which have high grain protein without a yield penalty, may achieve this by more efficient relocation of nitrogen from senescing tissues to grains, or by a more efficient uptake of nitrate and ammonia from the soil.

Where wheat is the major protein source for people the nutritional quality of grain protein becomes very important. The first limiting essential amino acid is lysine, so in

breeding programmes the major aim is to increase the amount of this amino acid. Unfortunately a negative correlation exists between lysine content and the protein content of grain. As the protein increases from 7-15% the lysine content falls from 4-3%. Increasing the protein concentration causes a significant increase in the ratio of storage protein to metabolic and structural proteins in the grain, the former being lysine deficient and the latter two relatively lysine rich. However, storage proteins are more digestible than structural proteins so for practical purposes it may be better to simply opt for increased protein content when seeking to improve the lysine content (Blackman and Payne, 1987).

2.1.2 Flour protein

The higher the protein percentage the better the quality expected for a given sample. In South Africa wheat with a protein content of about 12% and above is preferable (Koekemoer, 1997).

Near Infrared Reflectance Analysis (NIR), is used to measure protein and moisture contents, but can also be used to measure grain texture and to predict the potential starch damage. The reflectance energies of the different wavelengths are related to the physical and chemical nature of each sample. Multiple regression analysis is used to determine the relationship between reflectance energies of a test sample with known standards. Once calibrated the test samples can be analysed for several characters simultaneously in a 20 s period (Blackman and Payne, 1987).

2.1.3 Flour extraction

Milling properties are complex and may be split up, in relation to the breeding objectives, into percentage extraction of white flour, endosperm texture and water absorption. Judging milling texture by the appearance of the grain is often misleading because grains appearing flinty may actually be soft textured. Texture appears to be

simply inherited and there are a number of tests to measure this characteristic (Blackman and Payne, 1987).

Traditionally, vitreousness is associated with high-protein hard wheats, whereas opaque or mealy kernels are associated with softness and low protein content. The proportion of vitreous kernels has been used as an indication of kernel hardness (Eliasson and Larsson, 1993).

Hardness is highly heritable and wheat cultivars are specified either to be hard or soft. The harder durum wheats are used for pasta production, and the softest wheats are suitable for biscuits, whereas the wheats most suitable for bread-making have an intermediate hardness. The milling capacity as well as the flour yield will be higher with harder wheat than with softer wheat (Stenvert and Kingswood, 1977). Flour yield is related to kernel hardness. This is because of the easier separation of bran and endosperm in hard varieties (Eliasson and Larsson, 1993). Van Lill *et al.* (1995) reported that grains containing higher protein content were inclined to be harder, which in turn increased flour yield. Extraction is a function of hardness and the endosperm of hard firm wheat grains tend to separate more easily from the bran during the milling processes. More starch granules are damaged when hard wheat is milled, thereby improving water absorption (Bass, 1988).

Wheat conditioning is necessary to improve the physical state of the grain for milling and sometimes to improve the baking quality of milled flour. Conditioning involves adjustment of the average moisture content. This causes bran to toughen and become less brittle thus leading to better separation of the endosperm from the bran and it makes the endosperm more friable, less power is then required for grinding. Flour yield depends on how the endosperm separates from the bran when grounded. All the above-mentioned are related to the grain texture and wheat type (Eliasson and Larsson, 1993).

2.1.4 Breakflour yield (BFY)

Breakflour is the flour produced when the wheat is broken open in the first break system (Bass, 1988). Bran has a detrimental effect on loaf volume. However, the effect is related to the composition of the bran and the mill it comes from, as the method of separating the bran and the endosperm differs among mills. The coarser the bran fraction, the more detrimental its effect will be. The detrimental effect is attributed to a decrease in the gas holding capacity (Pomeranz, 1988).

2.1.5 Falling number (FN)

This is the effect of the α-amylase activity. Screening for this activity has a high priority in most breeding programmes, because the majority of wheat products are adversely affected by this enzyme. Selection for offspring with genetically controlled low levels of resistance to premature germination is difficult because of the large environmental component in sprouting and α-amylase production.

Several methods exist for measuring α-amylase activity, they include those of Farrand and Phadebas or determination of the Hagberg Falling number. The Falling Number (FN) method is widely used commercially, though it does not reflect the enzyme levels directly, it is sufficiently accurate for most purposes (Blackman and Payne, 1987).

The FN value represents the time in seconds required to stir a hot aqueous flour gel undergoing liquefaction in a viscometer and then allowing the viscometer stirrer to fall a measured distance through the gel (Kaldy and Rubenthaler, 1987).

Germinating wheat undergoes morphological and chemical changes whereby the carbohydrates are converted into complex sugar compounds by enzymatic activity. The α -amylase hydrolyses of starch reduces the viscosity of the suspension and thus increases the falling rate of the stirrer during the FN tests. This starch can be turned into a dextrin-like substance during baking. The water holding capacity is reduced, the

crumb weakened and made sticky (Blackman and Payne, 1987).. Flour with normal α -amylase activity and good baking quality has a FN value of 250 seconds or higher. Wheat with high α -amylase activity has a value of 65 seconds and produces sticky breads. High FN values in the range of 400 seconds indicate too low α -amylase activity for bread baking.

2.1.6 SDS-sedimentation (SDSS)

SDSS is a simplified water retention capacity test in the presence of lactic acid. Baking quality largely depends on the gluten proteins and the latter are caused to hydrate and swell by the lactic acid. Flour, water, and lactic acid are shaken together in a glass cylinder under specified conditions and the height of the sediment subsequently read. It has been shown that the sedimentation value is related to the granularity of the flour and that the sediment is an agglomeration of the course particles rather than the swollen protein. The sedimentation value is thus an indicator of hardness rather than of strength of the wheat (Lorenzo and Kronstad, 1987). This method is used for measuring relative gluten strength. Sedimentation values can range from 20 or less for low protein wheat of inferior bread-baking strength to as high as 70 or more for high protein wheat of superior baking strength. The high-protein helps to retain gas during fermentation, which results in higher loaf volumes (AACC, 1995)

2.1.7 Hectolitre mass (HLM)

The hectolitre mass is dependant on the kernel density and its packing efficiency. Hectolitre mass (HLM) is the mass per volume of wheat. HLM and 1000 kernel mass are the two parameters used as an indication of the flour yield after milling and are therefore an important selection criterion (Fowler and De la Roche, 1975). In South Africa a hectolitre mass of more than 76kg/hl is preferable (Francios Koekemoer, personal communication).

2.2 Yield

Yield remains one of the most important factors in wheat production (Jalaluddin and Harrison, 1989). Yield of cereals is composed of three components, namely the amount of spikes per unit area, the number of kernels per spike, and the individual kernel weight (Bulman and Hunt, 1988). Yield is affected by both the environment and the genotype, making it difficult to predict the harvest outcome (Fowler and De la Roche, 1975).

2.2.1 Thousand kernel mass (TKM)

In South Africa a thousand kernel mass (TKM) of more than 32g is preferable (Francios Koekemoer - personal communication). The weight of 1 000 counted kernels is determined, or the number of kernels is counted in a preweighed sample and the weight of the 1 000 kernels is calculated from it. The weight of 1 000 kernels can be corrected to a dry basis or any moisture basis. TKM can give the miller important information about the wheats' milling potential. TKM is one of the wheat quality parameters highly correlated with flour yield (Blackman and Payne, 1987).

2.3. Rheological characteristics

When bread ingredients are mixed in the correct proportions to make a dough, two processes commence: the protein in the flour begins to hydrate, i.e. to combine with some of the water to form a cohesive mass called gluten, which has peculiar extensible properties. It can be stretched like an elastic band, and possesses a certain degree of recoil or spring. Secondly, evolution of the gas carbon dioxide by the action of the enzyme in the yeast upon the sugars commences (Eliasson and Larsson, 1993).

2.3.1. Mixograph (MDT)

The quality of the final loaf of bread is strongly dependent on the mixing of each combination of flour and water. It is possible to find an optimum stage of dough development. The mixograph mixer measures the power used to mix the dough or, the resistance to mixing is recorded. The resulting mixing curve is described with such terms as dough development and breakdown. The more glutenins and the higher their molecular weight, the longer the development time will be. Breakdown starts after a decrease in the mixing curve is recorded. The rate of breakdown shows the stability of the dough and its sensitivity to mechanical treatment. The flour with the best baking performance has medium to medium-long mixing times. The aim of many rheological measurements is to find a way to differentiate between wheat varieties according to their baking performance without actually performing the baking test (Eliasson and Larsson, 1993).

Molecular weight distribution differs among wheat varieties, and strong wheat with medium-long mixing time contains more of the high molecular weight material. Moreover, these wheat varieties also contain more residual protein. It was found that fractions rich in low molecular weight (LMW) proteins decrease the mixograph developing time as well as the loaf volume in test baking (Tanaka and Bushuk, 1973). The fractions with a high proportion of high molecular weight (HMW) proteins, on the other hand, increased the mixograph developing time as well as the loaf volume in test baking. Such a relationship seems promising in the case of HMW glutenin subunits. These subunits are of greater importance for dough strength and dough stickiness than LMW glutenin subunits (Eliasson and Larsson, 1993).

Flour protein was reported to be negatively correlated to mixograph tolerance. Mixograph tolerance was independent of corrected or uncorrected loaf volume. Dough type is phenotypically correlated to all other characters except mixing tolerance (Souza *et al.*, 1993). Flours with medium to medium-long mixing times usually have good

mixing tolerance, good dough handling properties, and good loaf volume (Finney *et al.*, 1987)

The suggested mixing time in South Africa is 2 to 3 minutes, with 2.5 minutes as optimum (Francois Koekemoer – personal communication). A higher mixing time is not desirable, as apart from spending more time, the energy consumption is also higher.

2.3.2. Farinograph

It is not possible to make bread without water. Water is necessary for gluten formation, and water is the medium for all types of interactions and reactions that occur during the breadmaking process. The water content of standard bread dough is about 40%. However, the ingredients in the formula are usually expressed as a percentage of the flour by weight, and the water content in bread dough will then be around 65%. The optimum level of water addition is related to the composition of the flour. Both quantity and quality of protein influences water absorption (MacRitchie, 1984). Therefore it is necessary to determine this optimum level for each flour. This may, of course, be done in test baking, but it is more common to determine water absorption by the use of the Brabender farinograph, although it needs larger size samples than for most other tests and is a relative expensive apparatus (Finney *et al.*, 1987).

The farinograph measures and records resistance of a dough to mixing. It is used to evaluate water absorption of flours and to determine stability and other characteristics of doughs during mixing. The important factors are the absorption capacity, peak time, and the stability. In South Africa the absorption is suggested to be 60 as optimum but it can go up to 63 (Francios Koekemoer – personal communications). The water absorption of a flour is described as the amount of water necessary to bring the dough to a specified consistency at the point of optimum development. Absorption increases linearly with the amount of protein, but the slope of the regression line depends on the wheat variety. The rheological properties of a wheat flour dough are extremely sensitive to water content. It is evident that a decrease in the amount of water added

has a greater effect than an increase, at least within the range of water content (Eliasson and Larsson, 1993).

The flours from large wheat kernels had higher water absorption and a longer peak times than the flours from small and medium sized wheat kernels. Smaller wheat kernels showed greater mixing stability than the flours obtained from large and medium sized wheat kernels. The rheological variation among the flours from different sized wheats indicates the potential differences in their baking qualities. Uniformity of wheat kernel size plays an important role in milling stability (Blackman and Payne, 1987).

2.3.3. Alveograph

The alveograph was one of the first machines used to predict baking quality. It measures the resistance to biaxial extension obtained from a thin sheet of flour-water-salt dough (Bettge *et al.*, 1989). The dough prepared for use in the alveograph test needs to be stiff and have a low water concentration. The dough undergoes treatment similar to that of the baking process, by being sheeted, rolled, and moulded. It is moulded into a patty, which is then exposed to air pressure, forming a bubble. The alveograph records the pressure and time needed for the bubble to burst.

The interpretation of the alveograph results is much the same as that of the extensograph. The maximum curve height is an indication of the resistance and the length of the curve measures the elasticity. The resistance is influenced by the water absorption of the dough and the dough is developed with a constant increase of water added.

Randall *et al.* (1993) found the values of the alveograph (P, L and W) to be correlated with values obtained from the extensograph, but that only the P-value showed a negative correlation with flour protein content, wet gluten and loaf volume.

The P-value indicates the dough's ability to retain gas, the L-value is related to the dough's handling properties and its extensibility, while the W-value indicates the energy input needed to deform the dough. As with all the other rheological characteristics, protein content and composition have an influence on the alveograph.

2.4. Baking characteristics

2.4.1. Loaf volume (LFV)

This method provides a basic baking test for evaluating bread-wheat flour quality by a straight-dough process that employs short fermentation and in which all ingredients are incorporated in the initial mixing step. It is intended primarily for laboratory assessment of bread-wheat flour quality under vigorous fermentation conditions. Effects of ingredients and processing conditions, and particularly oxidation response, can also be assessed.

Baking is the final test of wheat quality as it indicates what the final product looks like. The desired higher loaf volume and good texture is a result of high protein content especially gluten in wheat grains. High protein flours with good quality are required for long fermentation baking methods, but low protein levels are tolerated for mechanically developed bread processes (Blackman and Payne, 1987). This also shows that there was no sprouting damage, as flour from sprouted wheat grains results in low loaf volumes and poor texture regardless of a cultivar being of good quality.

Strong flours must be used which develope an extensive viscoelastic matrix during dough formation, to retain the gas produced by fermentation. The dough expands and, after baking, a large well-aerated loaf is formed. If weak flours are used, loaves of small volume are produced which have poor crumb structure, being too firm and lacking resilience. Hard wheats are also preferred to soft wheats because their high water-absorption properties increase bread yield and resistance to staling (Blackman and Payne, 1987).

2.4.2. Baking strength index

Strong dough requires a high energy input to mix it to a consistency, which is optimal for breadmaking, whereas a weak dough requires little mixing. The difference is mainly caused by the protein quality and quantity. The stronger dough has a higher good-quality glutenin content, the protein complex that imparts elasticity. Whereas the weaker dough is deficient in glutenin, but exhibits extensibility imparted by the gliadin proteins (Blackman and Payne, 1987).

2.5. Protein quality

The quantity and the quality of flour protein largely determine bread quality. Quality is mainly controlled genetically while quantity is largely influenced by environmental factors (Peterson *et al.*, 1992) Protein quality is a major factor in determining whether a sample of wheat meets the required standard for potential dough development. Protein quantity is determined through assessing the nitrogen in wheat or flours. The nitrogen level is multiplied by 5.7 to approximate the protein content in flour. Near-infrared reflectance analysis of wheat has been developed as a means for fast protein quantification (Eliasson and Larsson, 1993).

2.5.1 Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Our understanding of the role of wheat proteins on baking is still incomplete, and two reasons for that are undoubtedly the complexity of their composition and their physical properties. Proteins can serve as markers for particular genes since it is the product of structural genes. Thus, from the proteins considerable information can be obtained about the chromosomes and the genome as a whole (Konarev *et al.*, 1979).

One of the techniques used to determine protein composition is gel electrophoresis, which separates protein in a polymer matrix on the basis of their apparent size, charge, and pH. The replacement of starch with polyacrylamide has made the formation of more reproducible gels with a wider variation in molecular sieving of proteins possible (Lookhart and Wrigley, 1995). The banding pattern of the proteins, as obtained from the electropherograms show only genotypic variations, so the environmental factors can be excluded to a large extent.

2.6. Storage proteins

With the original Osborne fractional extraction procedure five protein fractions were obtained: albumins (soluble in water), globulins (soluble in salt solutions), gliadins (soluble in aqueous ethanol), glutenins (soluble, or rather dispersible, in dilute acid or alkali), and an insoluble residue. According to the classification of Osborne, glutenins are the proteins remaining after the albumins, globulins, and gliadins have been extracted. Gluten is formed when wheat flour dough is washed to remove all soluble components and starch. Figure 2.1 illustrates this. The gliadins and glutenins are often described as the gluten proteins. Glutens constitute up to 50% of the total protein in wheat flour (Eliasson and Larsson, 1993).

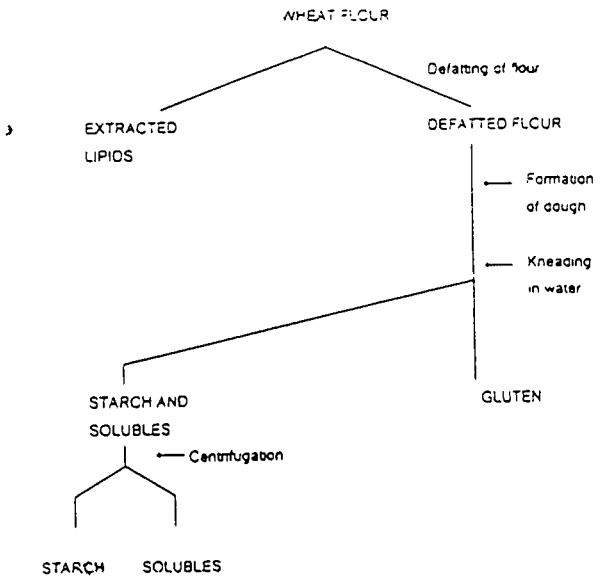

Figure 2.1: Separation of flour components and the definition of gluten (MacRitchie, 1984).

The composition of albumins and globulins does not vary between wheat varieties, and no correlation exists between the amount of albumins or globulins and baking performance (Eliasson and Larsson, 1993).

The introduction of better protein fractionation procedures, especially those separating in two dimensions, has made the identification of proteins determining good bread-making quality possible. Wheat gluten consists of two major protein types: gliadin, which confers extensibility to dough and glutenin, which confers elasticity (Gupta and Shepherd, 1990).

Glutenin has a much lower solubility than gliadins. It is virtually insoluble in 70% ethanol and only a portion of it dissolves in dilute acid solutions. It is built up from subunits into protein aggregates of high molecular weight, between 200 000 and 20 million Da. When glutenin is treated with reagents that dissociate disulphide bonds the subunits are released and fractionate by SDS-PAGE into two major groups, the high molecular weight and the low molecular weight subunits (HMW and LMW- subunits, respectively). There is wide variation amongst the varieties in the electrophoretic patterns of subunits (Wrigley *et al.*, 1996).

The chromosome location of the genes, which control the synthesis of gliadins and glutenin subunits, has been determined. Results suggest that there are nine major independently segregating loci for the gluten proteins. They are sited on the long and the short arms of group 1 and the short arms of chromosomes of group 6 (Payne *et al.*, 1984). It has been proposed that this allelic variation in protein type accounts for varietal differences in the quality of protein for breadmaking.

Genes on the long arm of chromosome 1 code for the HMW subunits, whereas the LMW-GS arise from linkage with gliadin genes on the short arm of chromosome 1 (Graybosch *et al.*, 1996). The amount of glutenins increases when the protein content of the wheat increases. An investigation of the amino acid composition of peptides obtained from glutenin revealed that the presence of an HMW peptide rich in glycine

(and glutamine and proline) could be correlated to good baking performance. The gliadins seem not to be crucial to the baking performance (Eliasson and Larsson, 1993).

It has been concluded that different combinations of storage protein variants that are present in the grain cause differences between varieties in protein quality for bread-making (Blackman and Payne, 1987).

2.6.1. Glutenin

After reduction, the glutenin protein can be divided into two groups by using electrophoresis, on the basis of their molecular mass: the HMW-GS (80-120kDa) and the LMW-GS (30-50kDa) (Graybosch *et al.*, 1996). Glutenins affect baking performance of wheat in at least three ways: through the molecular weight distribution, through the presence of certain HMW–GS and through the gliadin/glutenin ratio (Schepers *et al.*, 1993).

2.6.1.1. HMW-GS

Glutenins are polymers belonging to the polymeric prolamines (Shewry *et al.*, 1986). The molecular weight of these can extend into millions as it is the product of polymerisation of polypeptides through intermolecular disulphide bonds (Hamauzu *et al.*,1972). The HMW-GS consist of three structural domains: a nonrepetative sequence containing 3-5 cysteine residues at the N terminus: another nonrepetative sequence containing only one cysteine at the C terminus and in-between a number of repeated sequences of between 490-700 residues. The structures of the HMW-GS are similar to that of the ω-gliadins. The conformation is characterised by a large proportion of β-turns, which has been associated with the elasticity of glutenins, in the central domain (Tatham *et al.*, 1985). However, the HMW-GS differ from the gliadins in their higher glycine and lower proline contents (Shewry *et al.*, 1986).

The genes coding for the HMW-GS are found on the long arms of chromosomes 1A, 1B and 1D with their loci indicated as *Glu-A1, Glu-B1*, and *Glu-D1* respectively (Payne *et al.*, 1981). Each of these loci control certain bands or band combinations. The most significant of these bands are Glu 5+10 and Glu 2+12, both of which are coded for by genes on the D-genome. The HMW subunits 5+10 are said to be present in varieties of good baking performance and high sedimentation volume in the SDS sedimentation test, the inverse is true for subunits 2+12 (Lukow *et al.*, 1989). The consistent prominence of Glu 5+10 and Glu 2+12 among the HMW glutenin subunits is most striking and is consistent with studies on several other sets of wheats. It is significant that these proteins are associated with the D-genome, the one that distinguishes bread wheat from durum wheat. This explains why HMW-GS have not been found to be associated with dough properties in durum wheats (DuCros, 1987).

Payne *et al.* (1981) linked certain quality traits to specific bands, a value was assigned to each, with the highest score (10), indicating excellent baking quality. In Australian wheat the Glu-1 scores only accounted for 19% of the variation in bread-making quality of wheats, a much lower proportion than the 50-70% of variation attributed to this score in wheats from other countries. In some countries e.g. Australia the correlation between the 5+10 subunits and baking quality seem less (Campbell *et al.*, 1987). In South African wheat bands 13+16 and 17+18 were more prevalent than what was published for American, British, and Canadian wheats (Randall *et al.*, 1993).

2.6.1.2. LMW-GS

LMW-GS, unlike HMW-GS and gliadins, are not easily separated and analysed by one-dimensional SDS-PAGE or isoelectric focusing (IEF). The reason for the difficulty is that many of the LMW-GS overlap with gliadins (Zhen and Mares, 1991). This is not unexpected seeing that LMW-GS are controlled by genes found on the short arms of the group 1 chromosomes, which are closely linked to the genes controlling gliadins found on the same chromosomes (Rodriguez-Quijano and Carrillo, 1996).

This caused some confusion and Bietz and Rothus (1970) considered that some polypeptides may be common to both gliadins and glutenins, since the α, β, γ-gliadins and LMW-GS have similar electrophoretic mobilities and both are soluble in aqueous ethanol. This problem was resolved by the use of a two-dimensional electrophoresis, since LMW-GS had different positions to the α, β, ω-gliadins which indicated that they indeed were distinct proteins.

Despite the limitations of the one-dimensional SDS-PAGE system Payne *et al.* (1984) were able to map the genes coding for the b subunits. It has further been proved that each of the *Gli-1* loci, *Gli-A1*, *Gli-B1* and *Gli-D1* located on the short arm of chromosomes 1A, 1B and 1D, respectively, are closely linked to a locus coding for the LMW-GS (*Glu-3*). Examination of the banding patterns revealed that some bands were inherited simultaneously and formed combinations whilst others occurred as alternatives to each other, in the same cultivar (Gupta and Shepherd, 1988).

LMW-GS have been divided into two subunit groups, B (higher molecular weight, slower moving) and C (lower molecular weight, faster moving), subdivided into three groups (1-3). These subdivisions were further divided into patterns, indicated by letters. Group one consists of six combinations indicated by letters a-f. Genes on chromosome 1A control the few bands represented in these patterns. Group 2 was divided into nine pattern combinations (a-i); these patterns consisted of a lot more bands, with at least two or more B subunit bands. The combinations in group 2 are mainly controlled by genes on chromosome 1BS. Group 3 consists of five different combinations (a-e), controlled by genes on the short arms of chromosomes 1D. In this group (3) the banding patterns mostly constitute two bands from each subunit (Konarev *et al.*, 1979).

Despite the amount of information already available on LMW-GS, a few questions remain unanswered. This is due to the difficulties analysing the LMW subunit combinations. The bands in group 2, for example represent a wide range of mobilities, which overlaps with bands in group 1(*Glu-A3*) and group 3 (*Glu-D3*), and visa versa.

Thus the LMW-GS's effects on dough properties are largely unknown, although it is important in determining dough viscoelasticity (Eliasson and Larsson, 1993).

2.6.2. Gliadins

Gliadins are readily soluble in aqueous ethanol and consist of a complex mixture of polypeptides whose molecular weights range from about 30 000 to 70 000 Da as determined by SDS-PAGE (Bietz and Wall, 1972). Shewry *et al.* (1986) defined gliadins as monomeric proteins with intramolecular disulphide bonds, and that the conformations are thus stabilised by hydrogen bonds and hydrophobic interactions.

When fractioned by A-PAGE (acid polyacrylamide gel electrophoresis) they are subgrouped into α-, β-, γ- and ω- gliadins (Woychik *et al.*; 1961; Mosleth and Uhlen, 1990). The molecular weight of most gliadins are in the range 30 000-40 000 Da, with the ω- gliadins being larger with a molecular weight around 60 000-80 000 Da. There is considerable variation in gliadin-banding patterns between varieties, making it possible to use A-PAGE to identify varieties and varietal mixtures of grains (Wrigley, 1992).

Gliadins are inherited codominantly, with certain gliadins inherited as a block (Sozinov and Poperellya, 1980). This might be an indication that the gliadins inherited as a block are a cluster of structural genes (Wrigley, 1982).

The genes that synthesize gliadins are found on the short arms of chromosomes 1 and 6 respectively (Khelifi *et al.*, 1992). The genes found at the *Gli-A1, Gli-B1*, and *Gli-D1* loci on chromosome 1A, 1B and 1D respectively are referred to as the *Gli-1* genes. While those found at the *Gli-A2, Gli-B2*, *Gli-D2* loci of chromosomes 6A, 6B and 6D respectively are referred to as the Gli-2 genes (Rodriguez-Quijano and Carrillo, 1996; Jackson *et al.*, 1983).

In order to fully utilize variations in the gliadin-banding pattern to provide a means of identifying biotypes and cultivars or of possible influence on baking quality, a standard

nomenclature system is needed. The system most commonly used to analyse the banding patters, is a combination of the nomenclature used by Woychik *et al.* (1961) and that of Konarev *et al.* (1979). Gliadin zones were designated by a Greek letter as α, β, γ and ω (Woychik *et al.*, 1961). These zones contained bands and these bands were identified by numbers, this made this method more accurate (Konarev *et al.*, 1979). Additional adjustments were allowed to indicate deviations from the standard e.g. greater mobility (subscript 1), less mobility (subscript 2), higher intensity bands (underlined number), lower intensity bands (overlined number). Figure 2.2 gives an example of this system and Table2.1 shows this system in use.

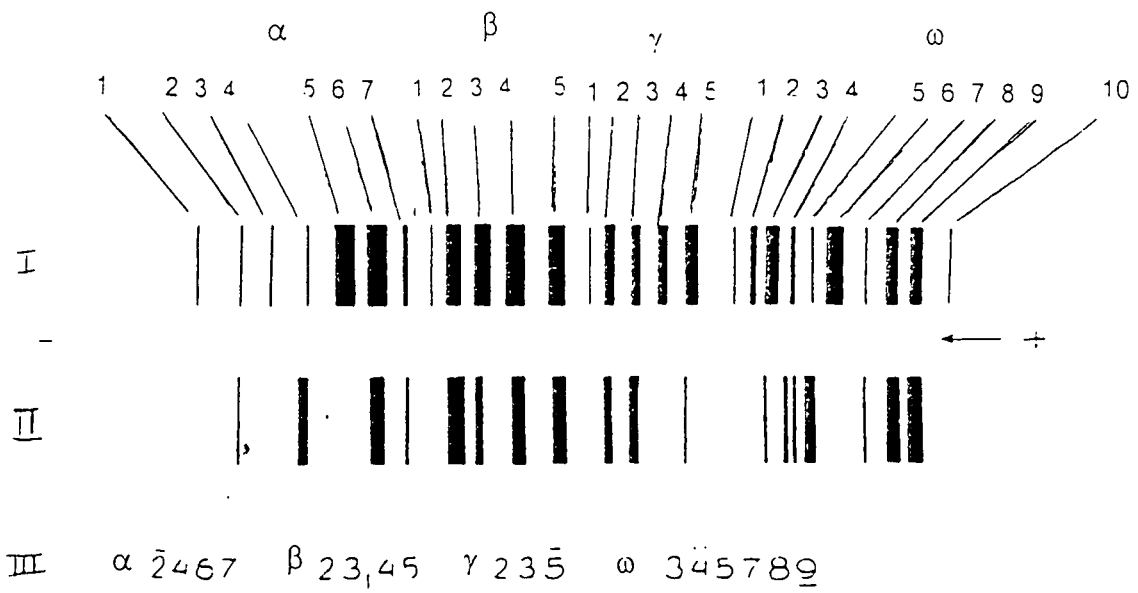

Figure 2.2. The standard spectrum of Gliadins and the formula used to identify cultivars (Konarev, 1979).

Table 2.1. A summary of the nomenclature system developed by Konarev *et al* (1979).

Gliadin zones and bands		Chromosome and its Arm
α	2	6A
	4	6A
	6	6D
	7	1B(S)
β	3	6B(S)
	4	6B(S)
	5	6B(S)
γ	2	1B(S)+6B(S)+1D(S)
	3	1D(S) +1A+1A(S)
	5	1A+1A(S)
ω	3	1B(S)
	4	1B(S)
	5	1B(S)
	7	1D(S)
	8	1D(S)
	9	1D(S)

Gliadins do not seem to be crucial to baking performance. When interchanged between wheat flours of different baking performances, the effect compared to that of glutenin is very minor, although groups of gliadins have been indicated to be related to endosperm hardness, dough strength, Chopin values, or Zeleny tests.

Gliadins indicated to be involved in flour quality are coded for by genes on chromosomes 1D and 1B. The gliadin bands most strongly associated with dough resistance in this study have not previously been studied, but they probably correspond

to components of the compound gliadin 34 of Wrigley (1982) These gliadins are presumably coded for by genes on the homologous group 6 chromosomes.

2.7. Resistance to eyespot in wheat

Eyespot, caused by *Pseudocercosporella herpotrichoides* (Fron) Deighton, is a widespread, serious disease of cereal crops in temperate climates, occurring in Europe, the USSR, South Africa (especially the Western Cape), parts of North America and Australia. The fungus has an anamorph (asexual: *P. herpotrichoides*) and a teleomorph (sexual; *Tapesia yallundae*) phase (Creighton, 1989).

Isolates of *Pseudocercosporella herpotrichoides* can be separated into two main types, W-type and R-type (Scott *et al.*, 1975; Creighton, 1989), according to differences in cultural characteristics and in host range. W-type isolates are more pathogenic to wheat than to rye, and form fast growing, even-edged colonies on the potato dextrose agar (PDA) whereas R-type isolates are equally pathogenic to wheat and rye. R-type isolates form slow growing, feathery or uneven edged colonies on PDA. Both W and R-type isolates possess heterothallic-mating systems with no evidence of sexual compatibility between the isolates of the two types (Dryer *et al.*, 1996). Based upon these findings it has been suggested that the two types be regarded as different species, *T. yallundae,* and *T. acuformis* for W and R-types respectively (Dryer *et al.*, 1996).

All South African isolates were identified as *Ramulispora herpotrichoides* (W-type). Results obtained, showed that although there is considerable genetic variation in the local population of *Ramulispora herpotrichoides,* the South African populations still share a high degree of similarity with overseas isolates. This indicates that genetic sources used for breeding resistant wheat cultivars in such countries can also effectively be employed in South Africa or vice versa (Cambell *et al.*, 1996).

Eyespot epidemics are unlikely to be limited to a shortage of inoculum since in many areas where winter wheat is grown conditions are favourable for sporulation. Viable spores are produced on dead organic material throughout the growing season. Moisture is required for infection to occur, since the mucilage must first be dissolved before the spores can be released into a spore suspension and become available for dispersal. Spores are normally dispersed from infected debris to plants of the new crop in rain-splash droplets. The spores are sticky and adhere to the leaves; these spores can thus not be removed by subsequent exposure to rain (Higgins and Fitt, 1984).

Wheat plants remain susceptible to eyespot throughout their growth cycle. Infection occurs on successive leaf sheaths and eventually the stem (Murray and Bruehl, 1986). Extracellular enzymes of the fungus degrade stem cell-wall materials weakening the stem. Symptoms are eye-shaped, elliptical lesions produced on internodes of the lower stem. Lesions are bordered by dark brown to greenish brown rings, have straw-coloured centres, and frequently develop on leaf sheath at soil level. Lesions may coalesce and lose their "eyespot" appearance. The fungus is limited to the basal areas of the plant. This results in lodging or dead stems that remain standing and form whiteheads (Murray and Bruehl, 1986).

Reduction in yield occurs only when epidemics become severe and cultivar and disease interaction occurs (Scott and Hollins, 1980). The effect of the disease on yield components is related to both host resistance and the genetic yield component of the cultivar. Yield losses of up to 50% can occur. Severe lesions may result in a lower 1 000-kernel weight, fewer kernels per head and tillers per square meter, and more lodging. Glynne *et al.* (1945) found that severe lesions reduced 1000-kernel weight but that the number of kernels per head was not affected, whereas Doussinault *et al.* (1983) found reductions in number kernels per head but not in 1000-kernel weight.

Scott and Hollins (1974) found that 1 000-kernel weight and the number of kernels per head was reduced on plants with severe lesions but that 1 000-kernel weight was more likely to be reduced than was the number of kernels per head on plants with moderate

lesions. Yield was indirectly related to the amount of lodging caused by strawbreaker foot rot and that the cultivars with resistance to *P. herpotrichoides* were unlikely to yield more than the susceptible cultivars unless they were also resistant to lodging (Scott and Hollins, (1974). Murray and Bruehl (1986) showed that compensation by individual tillers increased as the ratio of uninfected to infected tillers on a plant decreased.

There are three main ways to combat the disease and to reduce possible crop losses. The methods are: a) chemically, by using fungicides; b) biologically with the help of competitive fungi and c) genetically by planting resistant cultivars.

a) Chemical control

Fungicides are most commonly used to control disease infections. Some fungicides e.g. carbendazim, or prochloraz are only effective when applied at certain growth stages. In order to make the use of fungicides economically more feasible, growers in the UK were advised to apply fungicides only when severe disease infections are expected. A weather-based forecasting scheme has been developed for eyespot in wheat in West Germany (Fehrmann and Schödter, 1973). This scheme recommends that a fungicide be applied against eyespot after a period of 30-40 days with a high infection probability. Humidity and temperature were the most important variables. However subsequent weather conditions may result in severe eyespot developing when no spray was recommended resulting in fungicide being wasted (Bateman, 1987). The problem with late spraying is that the fungicide must penetrate the well-developed canopy to reach the infected area.

Another factor contributing to the severity of recent eyespot epidemics is the dramatic change in populations. Widespread resistance to the benzimidazole fungicides was found (Creighton *et al.*, 1989). Losses can increase since the disease is now largely resistant to the MBC and the benzimidazole fungicides, which previously gave effective control (King and Griffin, 1985: Yarham.1986).

b) Biological control

An alternative target is the pathogens saprophytic stage on straw. Isolate of *Pseudomonas fluorescens* and *Streptomyces griseoviridis* interfered with germination of *P. herpotrichoides* conidia *in vitro* and reduced disease severity. Studies were done to find other fungal antagonists capable of competing with the pathogen on straw, suppressing the inoculum production and the host infection. A *Trichoderma sp.* showed activity against both the R and W pathotypes of *P. herpotrichoides*. This fungus was the only one out of 24 tested that suppressed pathogen sporulation both on co-inoculated straws and on pre-inoculated straws (Clarkson and Lukas, 1993).

The occurrence of strains of the pathogen resistant to the benzimidazole-type fungicides (King and Griffin, 1985, Murray *et al.*. 1990) has led to renewed efforts to develope a disease resistant cultivars.

c) Genetical control

The level of resistance within the *Triticum* genus is generally too low to protect the plant from disease. However, unlike other fungal pathogens of wheat, there are few resistance genes for *P. herpotrichoides* available to wheat breeders (Murray *et al.*, 1994). There are four known sources of resistance to eyespot. The most effective resistance gene (*Pch1*) currently in use is derived from *T. ventricosum* Ces. a distant relative of wheat. This single gene, which is found in wheat line VPM-1, is located on chromosome 7D (Cadle *et al.*, 1997). Another resistance gene, *Pch1* is found on chromosome 7DV (Doussinault *et al.*, 1983; Mena *et al.*, 1992). Only *Pch1* has been extensively utilised in many breeding programme because of the linked isozyme and RFLP markers. *Pch1* does not provide complete resistance, and occurrence of new pathotypes of the pathogen that may circumvent this gene urged breeders to look for new resistance sources.

A homoeoallelic series of structural genes coding for endopeptidase on the long arm of group 7 chromosomes were reported to be associated with eyespot resistance (McMillin *et al.*, 1986; Koebner and Martin, 1990). The close linkage between *Pch1*,

the gene coding for resistance in VPM-1 and endopeptidase allele Ep-D1b has been used successfully in breeding programmes to select eyespot resistant genotypes (Allan *et al.,* 1989a). It was determined that RFLP Xpsr121 was closely linked to *Pch1* and Ep-D1b, by using Chromosome 7D recombinant lines (De La Pena and Murray, 1995). The readily scorable products of unique allele at the Ep-D1 endopeptidase isozyme locus can be used as a marker for resistance. Finding an isozyme marker is particularly beneficial, as screening is simple and rapid in contrast to present scoring methods. It is also less expensive and time-consuming than for example RFLP (Worland *et al.*, 1988).

A second, less effective gene, *Pch2*, is found in the cultivar Cappelle Desprez and is located on chromosome 7A. The origin of *Pch2* is not known (Cadle *et al.*, 1997). *Pch2* is not commonly used, primarily because the resistance conferred is inadequate to prevent yield loss in most years. Linkage relations between eyespot resistance gene *Pch2*, a gene encoding for an isozyme of endopeptidase, Ep-A1b and RFLP marker Xpsr121 on chromosome 7A were determined (De La Pena and Murray, 1995). Segregations of *Pch2*, Ep-A1b, and Xpsr121 fit an expected 1:1 single-locus ratio based on χ^2 tests. The order of these loci is *Pch2* - Xpsr121 – Ep-A1b.

Identification of markers more closely linked to *Pch2* than Ep-A1b would be useful in marker-assisted selection to develop eyespot resistant cultivars. Neither Ep-A1b nor Xpsr121 is suitable for selection of *Pch2* (De La Pena and Murray, 1995).

Neither *Pch1* nor *Pch2* confer complete resistance when used alone. Recently, Murray *et al.* (1994) demonstrated resistance in *D. villosum*, using a GUS tagged isolate and showed that the resistance was associated with chromosome 4V. After crossing 98 F2 plants, a ratio of 3:1 was obtained, indicating that a single gene, *Pch3*, determined resistance. This gene was located on the long arm of chromosome 4V, and closely linked RFLP's were identified. This genetic locus is not homologous with other known genes for resistance to *P. herpotrichoides* located on chromosome group 7 and thus represents a new source of resistance to this pathogen (Murray *et al.*, 1994). Mapping

and tagging of *Pch3* will enable breeders to combine all existing resistance genes into individual varieties with the goal of a more complete resistance.

The use of *D. villosum* (genome VV) in wheat improvement has been limited though, probably due to the fact that the V genome chromosomes do not pair well with wheat chromosomes (Sears, 1953). The extra effort required in gene transfer from *D. villosum* to wheat is warranted because of the small pool of resistance genes available for eyespot. The fact that a gene or genes determining resistance is located on a single chromosome 4V, increases the likelihood of a successful transfer to wheat. Efforts are now underway to introgress eyespot resistance gene(s) from *D. villosum* into adapted wheat genotypes for evaluation of resistance under field conditions (Murray *et al.*, 1994).

Probably the best levels of intra-specific resistance to the disease occur in the French variety Cappelle Desprez.The majority of the resistance present in this variety is carried on chromosome 7A with genes on chromosomes 1A, 2B and 5D modifying the levels of infection (Law *et al.*, 1976).

Resistance to eyespot conferred by genes on chromosomes 7A and 7D is not completely adequate in controlling eyespot and applications of fungicides are sometimes necessary (Hollins *et al.*, 1988). Even Rendezvous, which reportedly contains both the 7A and the 7D resistance genes (Hollins *et al.*, 1988), cannot sustain severe disease.

The discovery of a new genetic locus for resistance raises the possibility of combining multiple genes for resistance to eyespot and eliminating the need for fungicide applications. In addition to yield loss, pathogenic specialisation that could circumvent existing resistance genes and render them ineffective, is of concern (Scott and Hollins, 1980). As a result of specialisation to host species, the durability of eyespot resistance introduced into wheat from other species is questionable (Scott *et al.*, 1976; Scott and Hollins, 1977; 1980). However, a broader genetic base of resistance attained by

building a pyramid of multiple resistance genes into a single cultivar may prevent such changes in the pathogen and thus increase the chances of durable resistance (Murray *et al.*, 1994)

2.8. Amplified fragment length polymorphisms (AFLP)

Traditional selection methods require the infection and visual selection of resistant plants in the greenhouse or in field trails. These tests are vulnerable for variation in the environmental conditions and for level of infections. Marker assisted selection (MAS) would help reduce the need for time-consuming greenhouse and field trials. It will also reduce the number of individuals needed for testing, thus reducing the costs and size of breeding programme(Mohan *et al.*, 1997).

MAS- breeding is based on identifying close linkages between markers and the gene(s) of interest. The presence of the preferred gene is indicated by the identification of the presence of the marker. The marker must therefore be very closely linked to the gene to reduce the possibility of recombination and thus loss of the marker (Mohan *et al.*, 1997).

A few techniques are used presently to detect markers. Some of the more successfully used techniques are isozymes, restriction fragment length polymorphisms (RFLP), random amplified polymorphic DNA (RAPD), Sequence Tagged Sites (STS), Amplified Length Polymorphisms (AFLP) and microsatellites (Powell *et al.*, 1996).

Unfortunately the wheat genome is very complex and large, with low levels of genetic polymorphisms making the detection and analysis of markers very difficult (Bohn *et al.*, 1999). The genome also has large parts of repetitive sequences. In this study we are going to make use of the AFLP technique in wheat, and show how this technique has overcome some of the barriers.

AFLP is based on the detection of genomic restriction fragments by PCR amplification, and can be used for DNA's of any origin or complexity (Vos *et al.*, 1995). AFLP is sensitive to the quality of genomic DNA used. High- quality genomic DNA is necessary to ensure complete digestion by the restriction endonucleases, DNA that is not digested completely by restriction endonucleases can be identified by gel analysis (Lin and Kuo, 1995).

Fingerprints are produced without prior sequence knowledge, using a limited set of generic primers. The number of fragments detected in a single reaction can be determined/tuned by specific primer sets. AFLP is robust and reliable because stringent reaction conditions are used for annealing. The reliability of the RFLP technique is combined with the power of the PCR technique. This technique will display the presence or absence of restriction fragments rather than length differences. It resembles the RFLP technique with the major difference that it uses PCR amplification to detect the fragments instead of Southern hybridisation (Vos *et al.*, 1995).

Ideally a fingerprinting technique should require no prior investments in terms of sequence analysis, primer synthesis or characterisation of DNA probes. These methods are all based on the amplification of random genomic DNA fragments by arbitrary selected PCR primers. The patterns generated depend on the sequence of the PCR primers and the nature of the template DNA. PCR is performed at low annealing temperatures to allow the primers to anneal to multiple loci on the DNA. DNA fragments are generated when primer-binding sites are within the distance that allows amplification. One primer can give sufficient bands. These methods have a major disadvantage that they are very sensitive to the reaction conditions, DNA quality, and PCR temperature profiles, which limit their application (Vos *et al.*, 1995).

Polymorphisms detected with AFLP and RFLP assays reflect restriction size variation. AFLP polymorphisms results from DNA sequence variation at primer binding sites and from DNA length differences between primer binding sites. The recently developed

AFLP assay has generated considerable interest and appears promising for rapid identification and mapping of large numbers of markers. AFLP's generally have higher multiplex ratios than RFLP's. Of course, the multiplex ratio of the AFLP assay can be adjusted by altering the restriction enzymes chosen and the degree of 3'-nucleotide extension on the PCR primers, offering a high degree of flexibility to the experimenter. RFLP and AFLP are both capable of detecting single nucleotide mutations as well as insertions/deletions. Their sensitivity to these types of mutations is expected to vary, because each assay for polymorphism within differing lengths of genomic sequence and each exhibits its own sensitivity level of resolution for differences in band size. RFLP's allows exclusion of non-polymorphic bands from experimental consideration whilst AFLP does not allow it (Powell *et al.*, 1996).

AFLP's proved to be useful since it provides simultaneous coverage of many loci in a single assay and can be tuned to generate DNA fingerprints of the complexity required by altering the number of selective bases employed. In the U.K they are looking at the possibility of using AFLP's for measuring genetic diversity among wheat cultivars. It has an advantage over protein and other DNA techniques in that the polymorphisms seem to be less rare. During these studies they found some of the AFLP products to be organ-specific for a given species or genotype. The differential generations of AFLP products is a general phenomenon across three plant organs (seeds, leaves and roots) and in three plant species. The differential products are believed to arise as a result of DNA methylation differences between organs (Donini *et al.*, 1997).

Thus the organ type has a demonstrable effect on the fingerprint, at least where methylation-sensitive enzymes are used. It is therefore vital to use the same tissue when comparisons are done for phylogenetic studies. Seeds are convenient for this purpose since physiological uniformity is guaranteed. Unfortunately the chance of a fungal contamination of the template may increase, though this might not be enough to affect the fingerprint since a high proportion of contaminant is necessary to generate PCR product. Seeds give a more complex profile than DNA extracted from the roots and leaves, which can be a desirable attribute when more polymorphisms for marker

studies are wanted. AFLP can thus also be used to study spatial and temporal variation in DNA methylation (Donini *et al.*, 1997).

Chapter 3
The influence of eyespot resistance genes on breadmaking quality

3.1. Introduction

Eyespot is a widespread serious disease of cereal crops grown in temperate climates, such as the Western Cape region of South Africa. Wheat plants stay susceptible to eyespot, caused by *Pseudecercosporella herpotrichiodes* (Fron.) Deighton, throughout the growth cycle (Scott *et al.*,1975).

The name of the disease is derived from the eyeshaped lesions that form on the basal areas of the plant, after infection. The fungus invades the base of the stem, causing a weakening of the lower internodes, interrupting translocation and causing lodging or premature ripening and the appearance of whiteheads (Murray and Bruehl, 1986).

This leads to a reduction in yield, especially when the epidemic becomes severe (Scott and Hollins, 1974). Yield losses of up to 50% can occur, making this an economically important disease. Severe lesions result in a lower 1 000-kernel weight, fewer kernels per head and tillers per square metre, and more lodging. Scott and Hollins (1974) found that the 1000-kernel weight was more likely to be reduced than the number of kernels per head on a moderately infected plant.

A reduction in yield will, in turn, lead to a reduction in the income of farmers. This makes it very important to find sustainable protection for the crop from this disease. Three main ways of protection exists, reduction in crop losses can be obtained by chemical, biological and genetical control (Bateman, 1987; Murray *et*

al., 1990). With genetical control being the most effective and environment-friendly method.

A few sources of resistance have been found, with the most commonly used, a resistance gene derived from *A. ventricosum* Ces. This single gene, *Pch 1,* is located on chromosome 7D (Doussinault *et al.,* 1983). This gene does not, however, confer complete resistance, the search for resistance, thus continues.

Producing a crop resistant to eyespot is important, but the maintenance of the crop's quality and yield characteristics is even more important. Therefore, before breeders can decide to use a resistant cultivar in their breeding programmes the newly introduced gene's effect on the quality attributes must be tested.

In South Africa eyespot resistant cultivars have already been developed, but the effect of the resistance genes on the baking quality and the yield of the crop has not yet been tested.

The aim of this chapter was therefore, to determine whether the presence of eyespot resistance genes would have an influence on the baking quality of the NIL's.

3.2. Materials and methods

3.2.1. Isogenetic lines

Seed from near-isogenic resistant Palmiet and SST66 lines were obtained from the Small Grain Institute (SGI) breeding programme at Bethlehem. Table 3.1 contains a list of all the lines and the degree to which they were backcrossed. The susceptible parental lines were used as controls.

Table 3.1. A list of cultivars and NIL's used for the baking quality tests.

Entry	Name	Backcross	Generation
1	SST66 101	BC10	F5
2	SST66 102	BC10	F5
3	SST66 103	BC10	F5
4	SST66 105	BC10	F5
5	SST66 112	BC10	F5
6	SST66 130	BC10	F5
7	SST66 131	BC10	F5
8	SST66 132	BC10	F5
9	SST66 134	BC10	F5
10	Palmiet 202	BC8	F5
11	Palmiet 203	BC8	F5
12	Palmiet 207	BC9	F5
13	Palmiet 208	BC9	F5
14	Palmiet 211	BC9	F5
15	Palmiet 212	BC9	F5
16	Palmiet 213	BC9	F5

Palmiet and SST66 susceptible parents were crossed with the Roazon donor parent, and then repeatedly backcrossed to the susceptible parent. The Roazon donor parent contained the *Pch1* resistance gene, which was subsequently transferred to both the Palmiet and SST66 lines. Infections with the eyespot fungus was done to ensure the selection of only the resistant individuals.

3.2. 2. Experimental layout

The near isogenetic lines was multiplicated in the glasshouse at the University of the Orange Free State during the first part of 1999. The harvested seeds were weighed and 56g of seed of each line was used. The trial was planted on the ninth of June 1999, 25km west of Bloemfontein in the central Free State. A randomised complete block design, with four replications, was used. The plots consisted of two 5m rows spaced 48cm apart, with 7g of seeds planted per row. The planting was done by hand to ensure precision.

The plots were harvested and threshed, by hand, in November 1999. All the samples were cleaned individually before the yield components were determined. The quality analysis was done in the laboratories of the ARC-Small Grain Institute at Bethlehem. The Palmiet and SST66 parents were used as controls in all the procedures.

3.2.3. Characteristics measured

3.2.3.1. Quality characteristics

3.2.3.1.1. Flour protein content (FPC) AACC-Method 39-11

An infrared reflectance spectrophotometer was used. Calibrations were done using the Kjeldahl data. The protein reading is given as a percentage.

3.2.3.1.2. Flour extraction (FLY) AACC-Method 26-21A

This process is started by cutting the grain with a grain cutter to determine the kernel hardness (vitreous). This is then used to determine the seeds moisture content.

One kilogram of clean wheat grain is weighed and placed in containers. The required amount of water is added to each sample and the containers shaken to ensure uniform distribution of water. The tempered samples are allowed to stand in the closed container for the desired time. Tempering requires a minimum of 12 hours or can be done overnight for 18 hours at 16%moisture.

Adjustment and modifications of the settings of the mill (as directed in the AACC method 26-21A) should be done to ensure the anticipated flour yield. Flour yield should range between 70 and 75% depending on the wheat characteristics, cleaning feed rate, ambient conditions and maintenance of the mill. Warm the mill before use, and do a cleanout. The sample can now be milled. For the test samples remove the pans and record the flour weight for each pan (B1, B2, B3, C1, C2 and C3). Also take the bran pans at the back and weigh it. Weigh pooled bran and hand sieve it 20 times. Weigh the fine bran obtained and discard it. Flour obtained must be sealed in polythene bags in order to avoid moisture loss and absorption. It is very important to clean the mill in between the different samples.

3.2.3.1.3. Breakflour yield (BFY) AACC-Method 26-21A

The first three fractions of white flour, obtained during extraction, are referred to as breakflour yield. The flour obtained from the break rolls was determined as a percentage of the total flour regained. A Karee line was used as a control. A Bühler pneumatic laboratory mill was used for this purpose.

3.2.3.1.4. SDS-sedimentation

To determine SDS-sedimentation, weigh 20g of flour and determine the moisture content. Five grams of the weighed samples was placed in boats. Transfer the first sample to a calibrated 100ml cylinder. Shake up and down 10 times and place in water bath at 30°C. Repeat the shaking after 2 min. Shake again after 6 min and then add 50 ml reagent (15g/l sodium dodecyl sulphate and 0.9ml/l lactic acid) hold the cylinder horizontally and invert it left and right 5 times. Place back in the water bath. After 8 min repeat inversion again (5 times). Repeat inversion after 12 min for the third time. After a total of 18 min take the cylinder out of the water bath, put it on a level surface and take the reading.

3.2.3.1.5. Hectolitre mass (HLM)

The apparatus used consists of a standard quart kettle, balance and hopper with a round opening of 1.25 in diameter, and a stroker. Place sufficient grain in the hopper so that the kettle over-flows. Set the hopper over kettle with the outlet directly over the centre and allow grain to flow into kettle. Remove the excess grain by placing stroker on kettle, lightly jarring and stroking the grain with three full-length zigzag motions. Place the grains on a balance and record the weight.

3.2.3.2. Yield components

3.2.3.2.1. Thousand-kernel mass/weight (TKW)

A thousand healthy wheat grains were counted with the help of an automatic seed counter, and the mass determined.

3.2.3.2.2. Grain yield (T-HA)

Plot yield was determined for each entry and adjusted to ton/ha.

3.2.3.2.3. Heads per square meter (HSM)

The number of heads per square meter for each entry was determined.

3.2.3.2.4. Number of kernels per head (KPH)

Fifteen heads per entry were randomly picked, threshed and the seeds counted to determine the mean number of seeds per head.

3.2.3.3. Rheological Characteristics

3.2.3.3.1. Mixograph development time (MDT)

The mixograph measures and records the resistance of dough to mixing. The mixing curve indicates the optimum development time, tolerance to over mixing, and other dough characteristics and estimates water absorption.

Room temperature should be retained at ±20°C. Use 10 or 35g of flour with predetermined moisture and protein content. The water absorption at 12% protein content must be determined. Then the amount of water to be added must be determined. Start with two standard checks. Weigh the appropriate amount of flour into the mixing bowl. Create a triangular hole in the centre of the bowl and add the required amount of water into the hole. Immediately place the bowl into position and start the mixer and graph pen. Record the results for a period of 6 min. After the recording, allow the mixing head to run a bit to roll the dough.

Remove dough and bowl and clean thoroughly. The machine is then ready for the next sample.

3.2.3.3.2. Farinograph AACC-Method 54-21A

The farinograph measures and records the resistance of the dough to mixing. It is used to evaluate the absorption of flours and to determine stability and other characteristics of the dough during mixing. Values other than absorption are frequently derived from the farinograph.

Turn on thermostat and circulating pump at least one hour before the instrument will be used to assure that the temperature reaches 30°C. Start with two known standards. Weigh duplicates of 50g of flour for each sample. Place the first 50g of flour in the bowl, cover it and set the burette in the right front corner of the bowl. Turn on the chart paper. Place the pen at the 9 or 9.5 min position, turn the machine on at high-speed setting and wait till the pen reaches the zero minute mark. Then add 65ml of water. If the curve reaches the maximum and the 500 BU line is passed at the centre of the curve at peak point, leave the machine to proceed. However in most cases you will have to wait till the curve has reached the maximum and forms a bend. Now lift the pen and stop the machine. Bran can be added now. Mix the dough and bran.

The amount of water to be added or reduced can be calculated as follows: (#of blocks from curve centre to 500 BU) x 0.7.

If the curve did not reach the 500BU line then the amount of water must be reduced by the amount indicated by the calculations and vice versa.

Continue with the second 50g of flour. Repeat the steps mentioned above but now add the adjusted volume of water. When the graph has reached the peak point that depends on flour strength, leave it for an extra 12 min. The machine

can then be stopped and cleaned for the next sample. Determine the moisture content of flour as directed. Make an estimate of as-is absorption. Add flour and set pen at 9 min mark on paper.

Start mixer and run at 63rpm with dry flour 1min until zero min line is reached.

3.2.3.3.3. Alveograph

The alveograph measures resistance of dough to extension and the extent to which it can be stretched under the conditions of the method. A sheet of dough of definite thickness prepared under specified conditions is expanded by air pressure into a bubble until it is ruptured, and internal pressure in the bubble is graphically recorded on moving paper.

Determine the moisture content of 20g of test sample. Fill the burette with salt solution (12.5g NaCl/500ml) equal to the moisture content. Add the salt solution to the flour and simultaneously press the start button. Stop the mixer after one minute and scrape the dough from the sides, this ensures a uniform hydration. While the dough is mixing use an eyedropper and place drops of oil on the receiving plate, rolling frame, roller and resting plate. After 8 min stop the mixer. Reverse the direction of the kneader by pressing the extraction button. Start, cut the first 2 cm of dough strip off and discard it. Let the dough reach the notches on the extraction plate and cut off. Slide dough onto the stainless steel plate of the rolling frame. Sheet the dough by passing the roller over it for six times. Cut a dough patty out. Place dough patties on the oiled relaxing plates and place it at 25°C. Let it rest for 28 min. Transfer the patty to the alveograph. Slide the patty onto the fixed plate, replace the tamper and screw the ring to immobilize it. Flatten the patty by screwing the upper press in around twenty seconds without forcing. Remove the ring and tamper, start the alveograph. As soon as you see the bubble burst, stop the alveograph. Proceed in the same way for the rest of the dough patties.

3.2.3.4. Baking Characteristics

3.2.3.4.1. Loaf volume (LF) AACC-Method 10-09

This method incorporates all the ingredients in the initial mixing step. The method requires the following stock solutions:

Sugar salt solution

1090.9g sugar and 272.7g NaCl per 2l distilled water.

Yeast suspension

353.33g compressed yeast per 2l distilled water.

Ascorbic acid solution to give 40-50ppm/100g flour in 5ml.

0.40-0.50g ascorbic acid/ 500ml distilled water.

$KbrO_3$

40.0g/2l distilled water

Preheat fermentation cabinet and oven. Warm dough mixer up and set temperature to ensure that when mixed the dough will reach a temperature of 29°C.

Place bowl on mixer and set the automatic timer. Start mixer; avoid placing the salt on top of the yeast. Determine the dough's minimum point of mobility. Mix to optimum and record total mixing time. Transfer dough from bowl to table top, measure the temperature. Round dough by hand, keeping the smooth skin on top. Place seem side down in lightly greased pan, and return to fermentation cabinet. After 52 min the dough is ready for the first punch. The dough is taken from the pan and placed onto lightly floured surface. Then it is passed through the sheeter lengthwise. The sheeted dough is then folded in half twice, and replaced, crease side down, in pan and fermentation cabinet. The second punch follows 25 min later exactly the same way as the first. The dough is than moulded either mechanically or hand powered. Place seem side down in lightly greased pan, and return to fermentation cabinet. Proof to required height

(usually 33-35 min). Bake 100g loaves at 215^{0}C for 24 min. Measure the volume by rapeseed displacement and hour after baking. Place in wax or plastic bag for external and internal scoring.

3.2.4. Statistical analysis

The analyses of variance and the coefficients of correlation were determined using AGROBASE '98 (Agronomix software Inc., Winnipeg, Canada).

3.3. Results

An analysis of variance was performed on all the milling, rheological, baking and yield characteristics for all the lines. In all the cases, comparisons were done between the susceptible parental line of each cultivar and the resistant progenies. Table 3.1 contains a summary of the ANOVA results obtained for all the traits tested.

Table 3.2 Summary of the analysis variance (ANOVA) performed for the measured characteristics.

Trait	MSE	Pr>f	Grand mean	C.V.	LSD	S.E.D.	h
Yield	9677.11	0.0751	273.659	19.18	61.2089	72.7384	0.143
TKM	2.21388	0	39.595	3.76	2.8091	1.0521	0.646
HLM	3.33203	0.0325	80.414	2.27	3.4463	1.2907	0.18 9
# Grains	0.68312	0.6384	41.158	11.05	8.5862	3.2158	
# Heads	154.672	0.1866	205.513	16.53	64.1539	24.0278	0.082
SDSS	7.48148	0.0076	85	6.17	9.8972	3.7069	0.255
BFY	0.32761	0	22.929	2.5	1.0806	0.4047	0.974
FLY	0.79633	0	73.091	1.22	1.6848	0.631	0.569
FPC	2.15538	0.7203	14.487	10.13	2.7718	1.0381	
MDT	0.05675	0.5559	1.77	13.46	0.4498	0.1684	
LFV	16.6058	0.12	011.118	2.25	42.9115	16.0718	0.115
LFV12	816.606	0.7756	911.645	6.78	116.636	43.6841	
PLR	0.00337	0	0.276	21.03	0.1096	0.0411	0.757
ALVEOW	33.8031	0.0664	197.908	10.52	39.3224	14.7276	0.151
Strength	0.14123	0.0402	30.341	10.5	6.0123	2.2518	0.178
arinoB	0.69504	0	58.097	1.43	1.574	0.5895	0.997

Degrees of Freedom (DF) =75
Entries DF = 18
Error DF = 54
P=0.01

MSE = Mean sum of error
C V. = Coefficient of variance
LSD = Least significant differences
S E.D = Standard error of deviation

= Number

h = Heritability

TKM = Thousand kernel mass, HLM = Hectolitre mass, SDSS = Sodium dodecyl sulphate sedimentation, BFY = Breakflour yield, FLY = Flour yield, FPC = Flour protein content, MDT Mixing development time, LFV = Loaf volume, LFV12 = loaf volume at 12%, PLR = P/L ratio, ALVEOW = Alveograph

The following results were obtained when comparisons were done in groups, between the parents and the progeny. The averages for the four replications of comparisons between the parental lines and crosses, are summarised in Figures 3.1 to 3.45

3.3.1. Milling characteristics

3.3.1.1. Flour protein content

The Palmiet susceptible parental line was ranked third, with Palmiet lines 202 and 207 having higher values. Palmiet line 208 gave the lowest value (13.38), but none of the differences were significant. This indicates that none of the lines had a value lower than the 12% that is acceptable (Fig 3.1).

Line SST66 134 (15.75) has the highest value, with the parental line ranked sixth. Line 112 gave the lowest value, but no significant differences existed between the lines (Fig. 3.17).

The effect of blocks was significant (p=0.01), which suggested that there were large differences between blocks. The effect of entry was not significant, which indicates that there were no large differences between entries.

3.3.1.2. Flour extraction

Palmiet lines 213 (72.72) and 202 (72.48) gave the highest values, respectively, with the Palmiet parental line ranked third. The progeny did not differ significantly from the parental line. Palmiet line 207 (70.81) gave the lowest value of all the Palmiet lines and was ranked eighth (Fig. 3.2).

SST66 line 131 gave the highest value while lines 102 and 122 ranked second and third respectively. The SST66 parental line gave the seventh highest value (74.01), with SST66 line 132 the lowest value (73.68). It is clear from the close range of the values that the differences were not significant (Fig. 3.18).

The heritability average (56.9%). Both the values for the block and entry are lower than the p-value, indicating that both the environment and the genotypes of the lines plays an important role in the differences that exists between the lines. The averages ranged from 70-74% indicating successful extractions.

3.3.1.3. Breakflour yield

Only Palmiet lines, 207 and 211 were harder than the susceptible Palmiet parent. The Palmiet lines with the highest values were line 202 (19.59), line 212 (19.07) and line 213 (19.06). The differences observed were not significant (Fig. 3.3).

The same held true for the SST66 cultivar. Two lines gave lower values than the SST66 parental line. These SST66 lines were 131 and 112 (25.29). The lines with the best performance were lines 132 (26.44), 103 (26.20) and 105 (25. 94). As shown in Figure 3.3 the differences observed were not significant.

The heritability for this trait was high (97.4%). The effect of both blocks and entries were significant, suggesting large differences between blocks and between entries.

3.3.1.4. SDS-sedimentation

Sedimentation quality values can vary from 20 or less for low protein wheat of inferior bread-making quality to as high as 70 or more for high protein wheat of superior bread-making quality. The high protein helps to retain gas during fermentation, which results in higher loaf volumes.

Five of the seven Palmiet progenies have higher values than the Palmiet parental line. The two lines with a lower SDSS value were Palmiet lines 213 and 212, although none of the differences were significant. The averages ranged from 77.25 (212) to 82.25 (208) (Fig. 3.4).

The SST66 susceptible parental line had the second lowest value with an average of 85.50, and thus ranked tenth. The only line with a lower value was

131. The differences were not significant. The line with the best value, averaging 90.75 was SST66 line 132 (Fig. 3.20). SST66 lines 112 and 134 ranked second and third respectively. The heritability for this characteristic was low (25.5%).

3.3.1.5. Hectolitre mass

The heritability was very low (18.9%). No significant differences were observed between the Palmiet lines. The Palmiet parental line ranked fifth, only Palmiet lines 207, 203 and 201 gave lower values. Palmiet 213 gave the highest and Palmiet 201 the lowest value (Fig. 3.5).

The susceptible SST66 parent ranked third of all the SST66 lines, with lines 102, 122 and 132 with a higher HML values. SST66 line 134 gave the lowest value (77.06). The average values ranged from 77.6 to 80.32. None of the differences were significant (Fig. 3.21).

All the lines had a HLM higher than the preferable value (76kg.h^{-1}) for South African cultivars.

3.3.2. Yield components

3.3.2.1. Thousand-kernel mass (TKM)

The susceptible Palmiet parental line ranked third. All the progeny except Palmiet 203 and 202 gave lower TKM values than the parental line, though the differences were not significant. The averages ranged from 39.90 (213) to 44.50 (203). The highest value was obtained by Palmiet line 203 and the lowest by line 213 (Fig. 3.6).

The SST66 susceptible parent gave the best value, when compared to the SST66 progeny. The only significant difference was found between the parental line and lines 101 and 134. The average values for the SST66 lines ranged between 36.30 (134) and 40.10 (parent). The heritability of TKW was 64.6% (Fig. 3.22).

3.3.2.2. Grain yield

Palmiet 213 (1532.08) gave the highest yield of the Palmiet lines though the difference between the parental line and progenies were not significant, except for line 202. Palmiet line 202 (1336.15) gave the lowest yield of all the lines. The susceptible parental line gave the third best average (Fig. 3.7).

No significant differences were observed between the SST66 lines. The progeny, with the exception of SST66 130 (1071.10kg/hl), gave, on average, better results than the susceptible parent. The parental line ranked tenth. SST66 line 112 gave the highest (1337.80kg/hl) and line 130 the lowest values (Fig. 3.23).

The heritability of yield was very low (14,3%). The block effect was significant, indicating large differences between replications. The differences between entries were not significant.

3.3.2.3. Heads per square metre

The number of heads per square metre for each entry was determined. Both the entry and the block has values higher than 0.05 which shows that no significant differences exist between the lines and that again the environmental role can not be disregarded. The heritability was very low (8.2%) which can be seen as an indication of the genotype environment interaction. This indicates that the environment plays an important role.

Palmiet line 202 gave the highest value (229.50), with the parental line ranked fifth. Palmiet lines 212 and 211 had lower values but none of the progenies differed significantly from the parental line. (Fig. 3.8)

In the SST66 cultivar, line 105 gave the best value (253.0) and was the only line that differed significantly from the parental line (185.25). Line 102 gave the lowest value (180.75) (Fig. 3.24).

3.3.2.4. Number of kernels per head

The block effect was significant, indicating large differences between replications. The effect of the entry was not significant, although this was not reflected in the actual values.

The averages had a wider range of dispersion. Palmiet line 208 gave the highest value (43.55) with line 202 as the lowest (37.08). The Palmiet parent ranked fourth, but the differences were not significant. (Fig. 3.9)

There were no significant differences between the SST66 lines. Lines 105, 112 and 130 had less kernels per head than the parental line, which ranked seventh of all the SST66 lines (Fig. 3.25).

3.3.3. Rheological characteristics

3.3.3.1. Mixograph development time

Only Palmiet line 202 (2) gave a mixing time in the range of the acceptable. The Palmiet parental line ranked second with a mixing time of 1.98 min and line 203 third. The Palmiet line with the lowest mixing time was 212 (1.78), though the values did not differ significantly (Fig. 3.10).

SST66 line 134 had the longest mixing time (1.85 min). The parental line was ranked fifth with a time of 1.70 min and line 112 had the shortest mixing time. The difference between the parental line and the progenies was not significant (Fig. 3.26).

3.3.3.2. Farinograph

Palmiet line 212 had the highest water absorption rate (64,85) with line 203 the lowest (61.40). There was not enough seed of Palmiet 207 to do this test. The Palmiet parent ranked fourth and a significant difference existed between the parental line and line 212. No other significant differences existed between the Palmiet lines. (Fig. 3.11)

The SST66 susceptible parent gave the best results (61.67), while lines 131 and 103 ranked second and third respectively. SST66 line 102 gave the lowest absorption value (59.10%). This was also the only line with an absorption value lower than the ideal (60-63%) for South African cultivars. No significant differences existed between entries (Fig. 3.27).

The heritability for this characteristic was high (99.7%) indicating that the genotype plays an important role in this characteristic. The effect of both blocks and entries were significant, which indicates that there were significant differences between the blocks and the entries.

3.3.3.3.1. Alveograph (P/L ratio)

Palmiet line 212 (0.44) had the best results with lines 203 and 211 second and third respectively. Palmiet parental line ranked fourth (0.41) and line 208 (0..32) gave the lowest value. The difference was not significant, though all the values were significantly lower than the preferred P/L ratio of 0.8 (Fig. 3.12).

SST66 parental line gave the highest P/L value with lines 103 and 102 second and third respectively. Line 112 (0.16) had the lowest value. No significant differences existed between the lines (Fig. 3.28). The heritability of this trait was high (75.5%).

3.3.3.3.2. AlveoW

Palmiet parental line (206.50) ranked third with lines 202 and 203 giving higher values. Palmiet line 208 (188.25) gave the lowest value, but none of the differences were significant (Fig. 3.13).

SST66 parental line (173.50) gave the lowest value. Line 134 (209) gave the highest value with lines 101 and 103 second and third respectively. No significant differences were found between the lines (Fig. 3.29).

3.3.4. Baking Characteristics

3.3.4.1. Loaf volume (at 12%)

Palmiet line 208 (957.5) gave the highest volume with line 213 second and the parental line (913.25) third. Line 202 had the lowest volume; none of the differences were significant (Fig. 3.14).

SST line 112 (950) gave the highest volume, with the parental line (934) second and line 101 third. SST66 line 134 (865) gave the lowest volume. The differences were not significant (Fig. 3.30).

3.3.4.2. Loaf volume

Palmiet line 213 (1002.50) had the highest volume , followed by Palmiet line 207 (1001.25) and the parental line (1001.25). Line 211 had the lowest volume (981.25). The differences were not significant (Fig. 3.15).

SST line 101, line 130 and the parental line had similar volumes of 1030.00. SST line 131 had a lower volume (1025), with SST 66 line 105 having the lowest volume (1010). The differences were not significant (Fig. 3.31).

3.3.4.3. Baking strength index

The susceptable Palmiet parent line(31.57) ranked third with lines 202 and 203 giving higher values. Palmiet line 208 (28.78) gave the weakest dough, but the differences were not significant (Fig. 3.16).

SST 66 parental line (26.53) gave the lowest value. The only significant difference was found between the parental line and SST 66 line 131 (33.46), which gave the highest value. SST 66 lines 101 and 103 were ranked second and third respectively (Fig. 3.32).

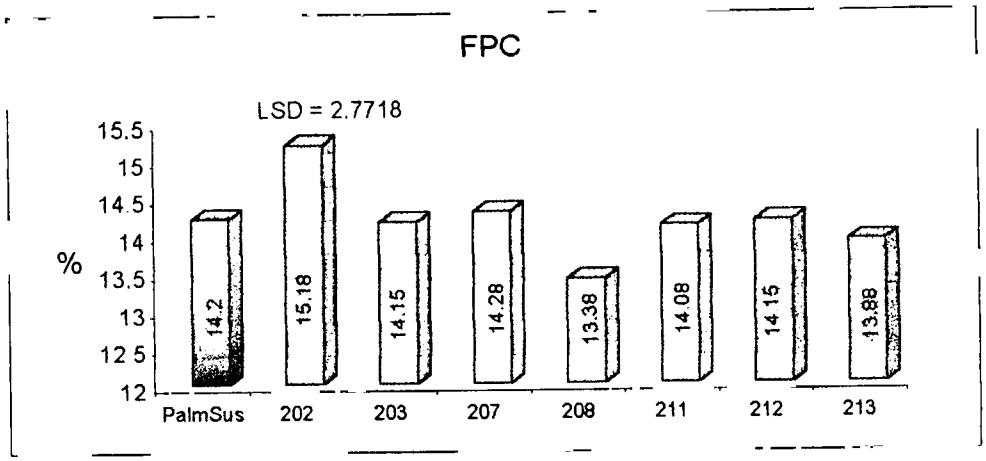

Figure 3.1 Flour protein content of Palmiet and its NIL's

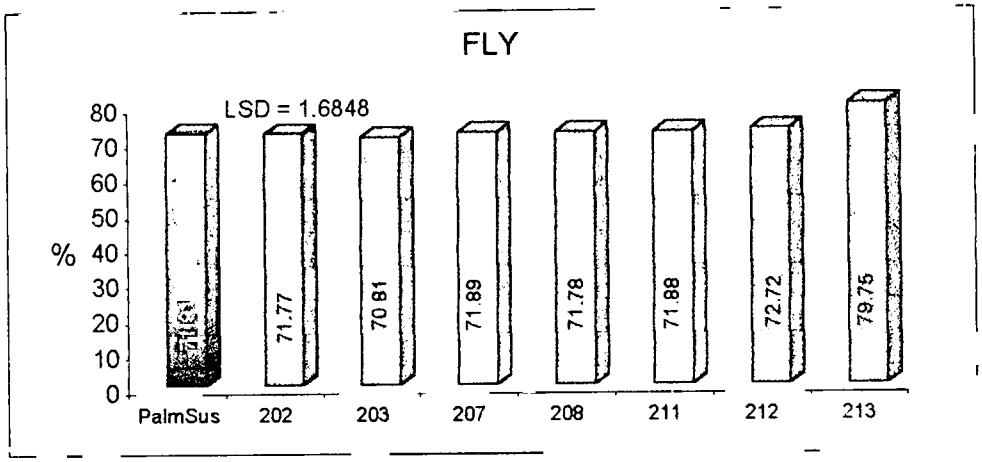

Figure 3.2 Flour extraction of Palmiet and its NIL's

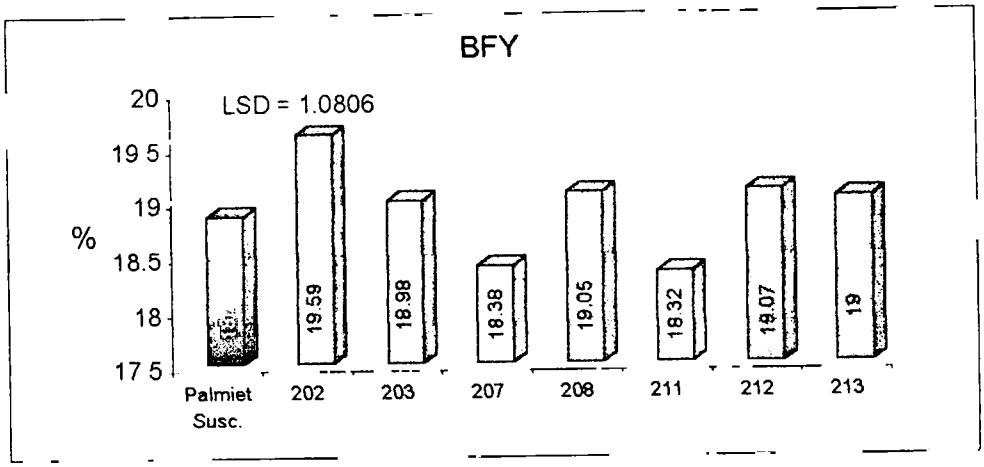

Figure 3.3 Breakflour yield of Palmiet and its NIL's

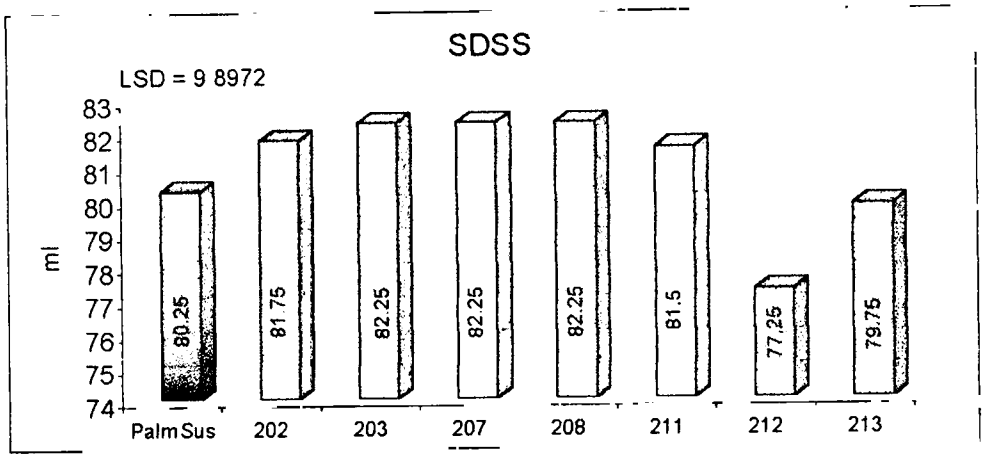

Figure 3.4 SDS–sedimentation test of Palmiet and its NIL's

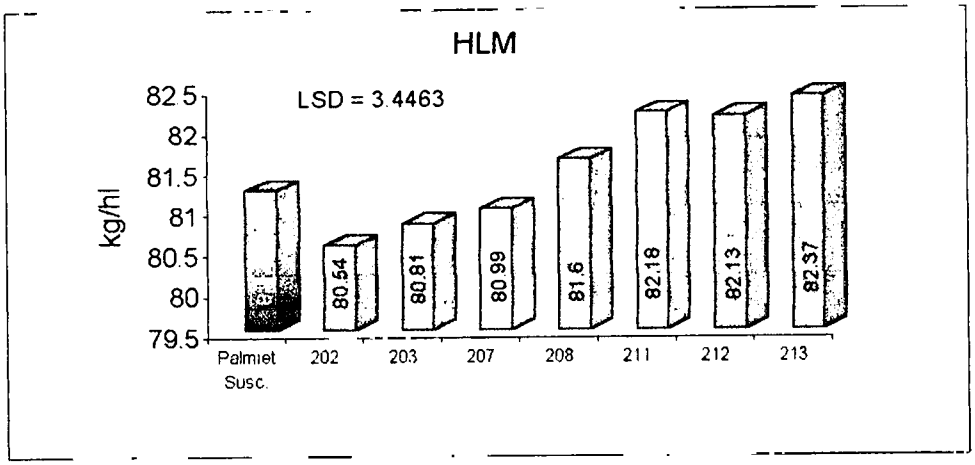

Figure 3.5 Hectolitre mass of Palmiet and its NIL's

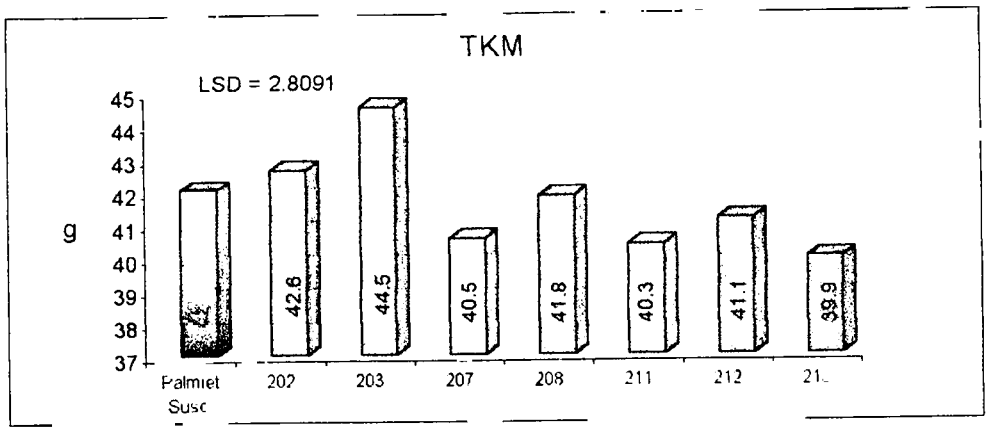

Figure 3.6 Thousand kernel mass of Palmiet and its NIL's

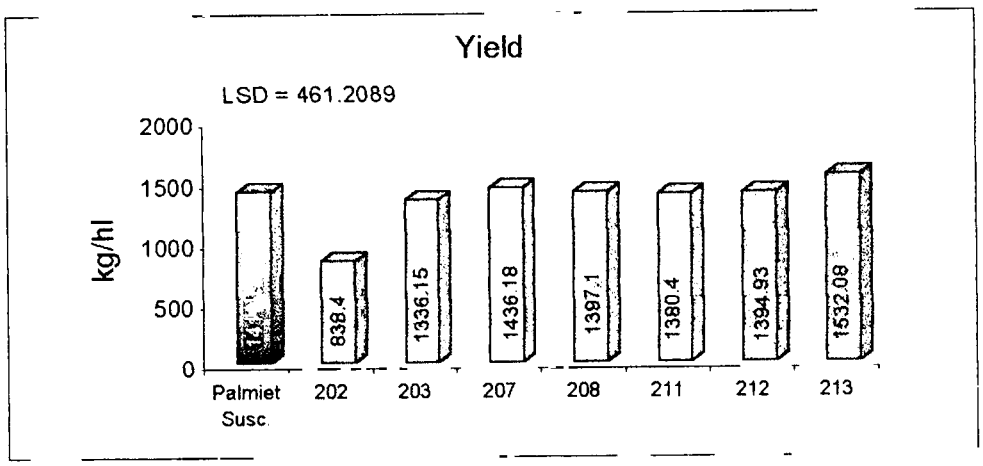

Figure 3.7 Yield per plot of Palmiet and its NIL's

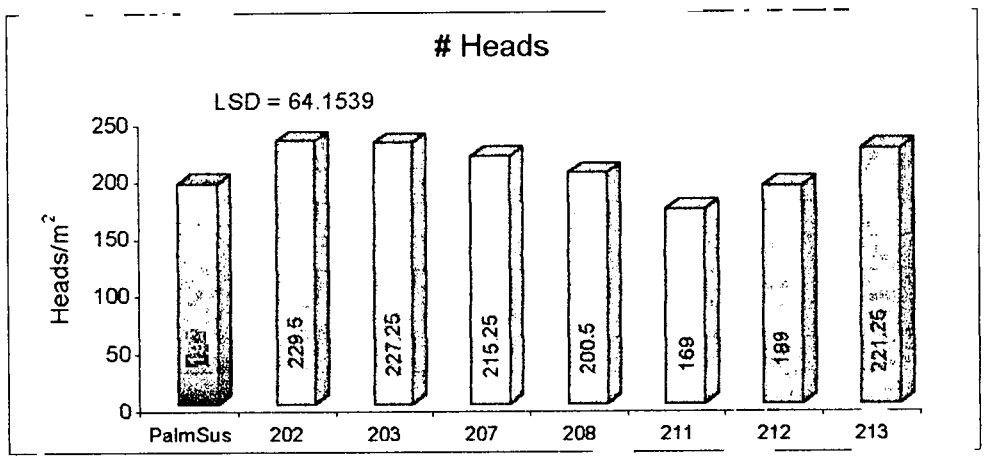

Figure 3.8 Number of heads per square metre of Palmiet and its NIL's

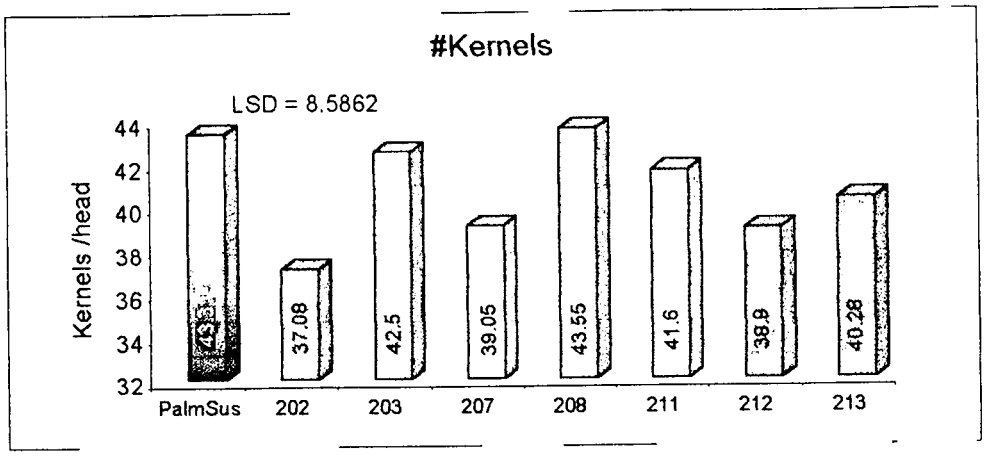

Figure 3.9 Number of kernels per head of Palmiet and its NIL's

Rheological Characteristics

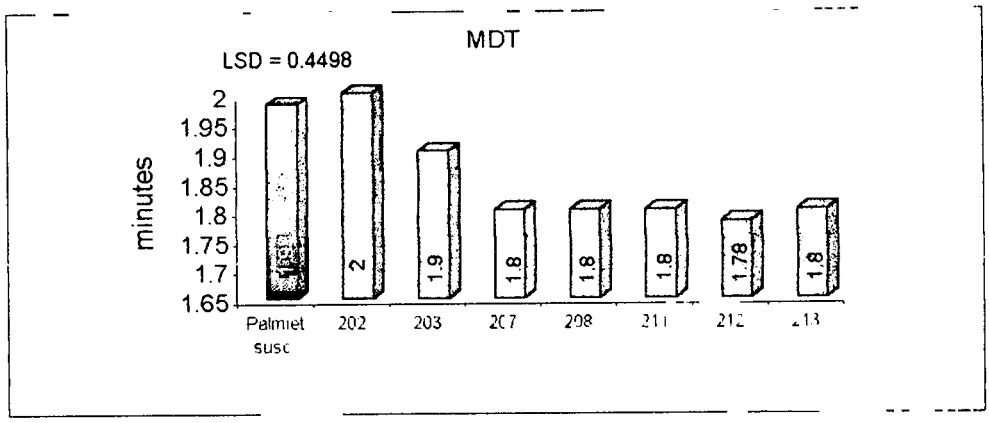

Figure 3.10 Mixograph mixing time of Palmiet and its NIL's

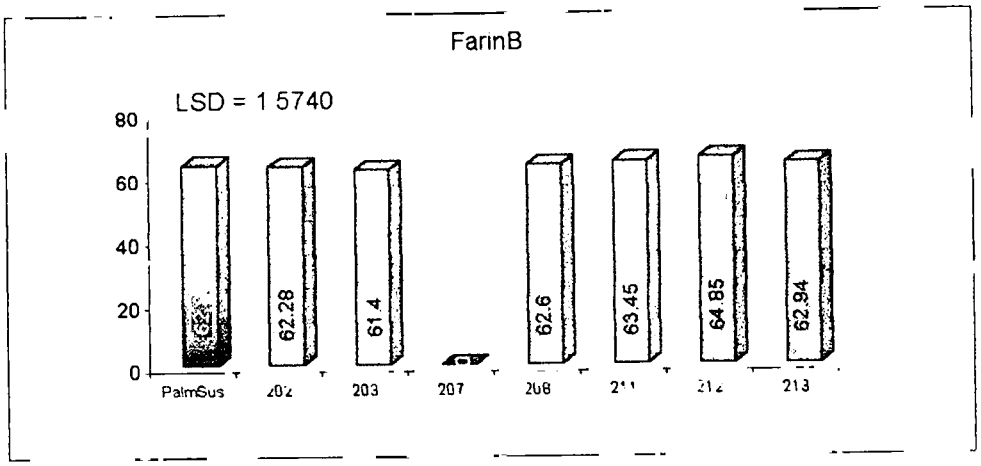

Figure 3.11 Farinograph water absorption of Palmiet and its NIL's

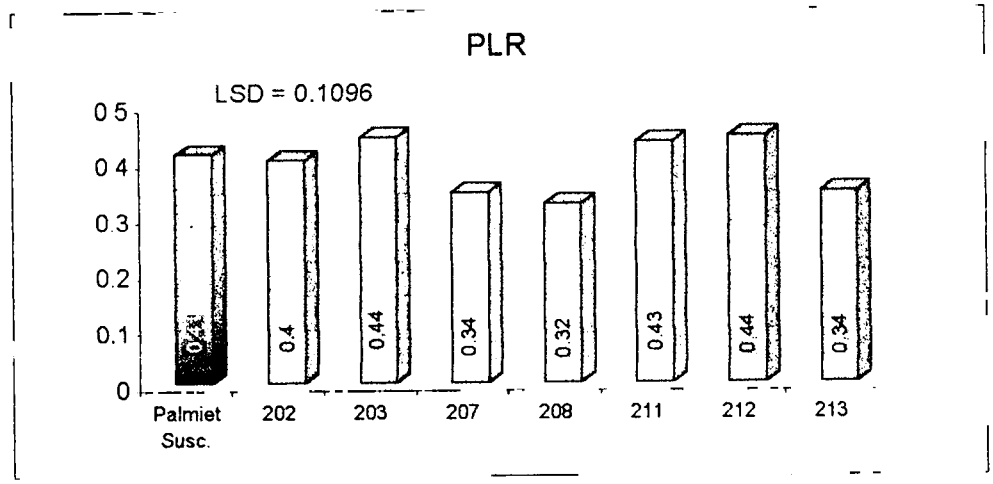

Figure 3.12 Alveograph P/L ratio of Palmiet and its NIL's

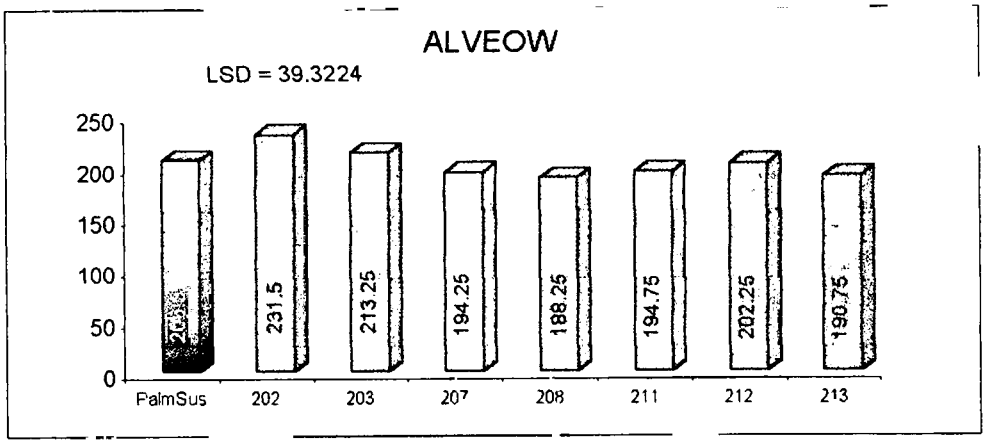

Figure 3. 13 Alveograph W-values of Palmiet and its NIL's

Baking characteristics

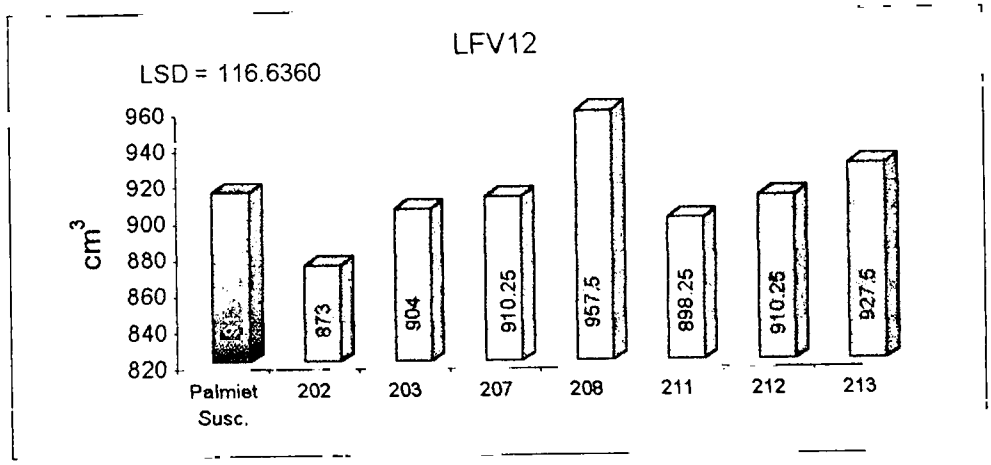

Figure 3.14 Loaf volume at 12% of Palmiet and its NIL's

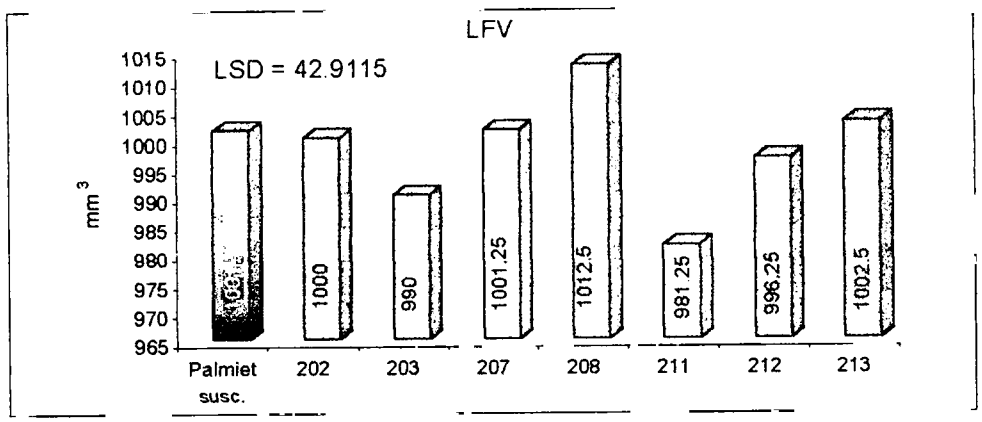

Figure 3.15 Loaf volume of Palmiet and its NIL's

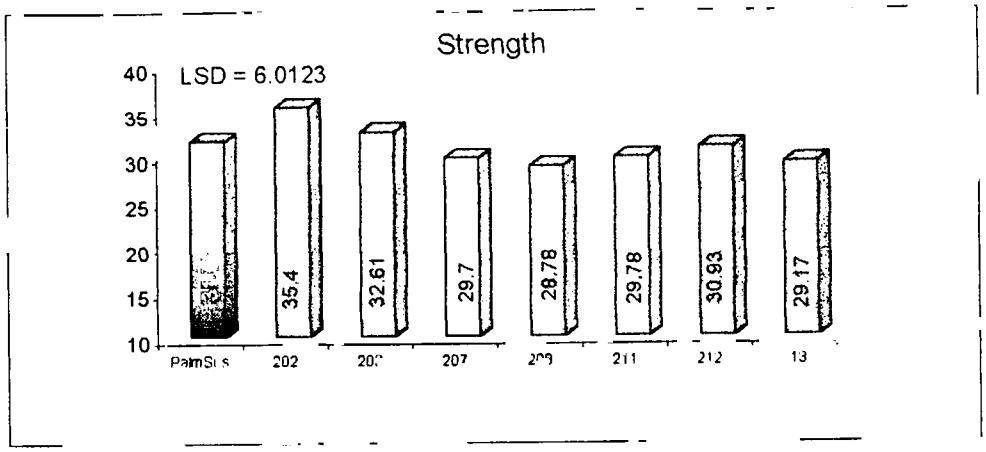

Figure 3.16 Baking strength index of Palmiet and its NIL's

Milling Characteristics of SST66

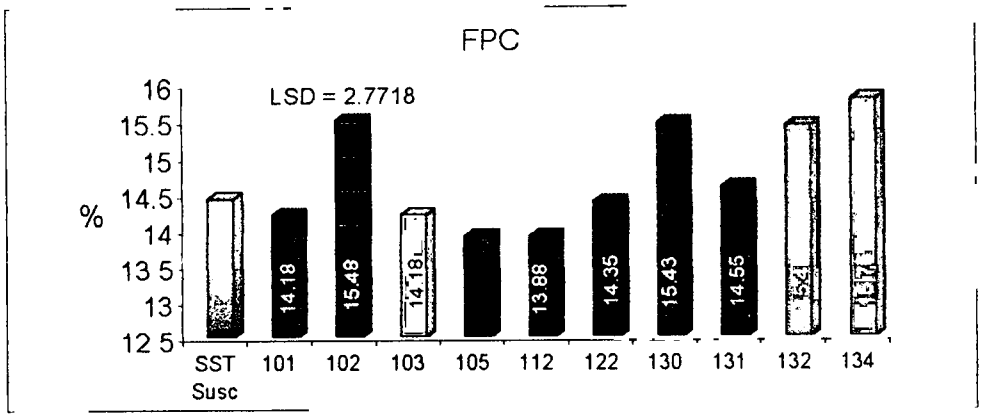

Figure 3.17 Flour protein content of SST66 and its NIL's

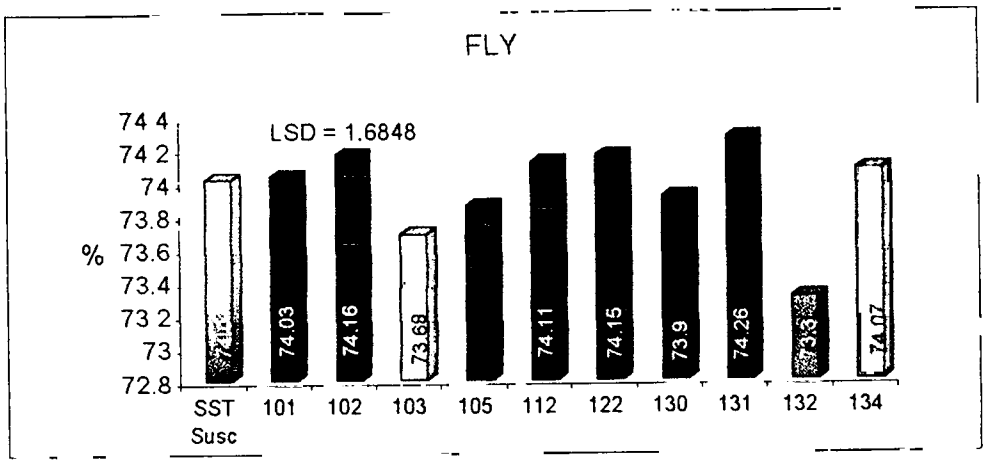

Figure 3.18 Flour extraction of SST66 and its NIL's

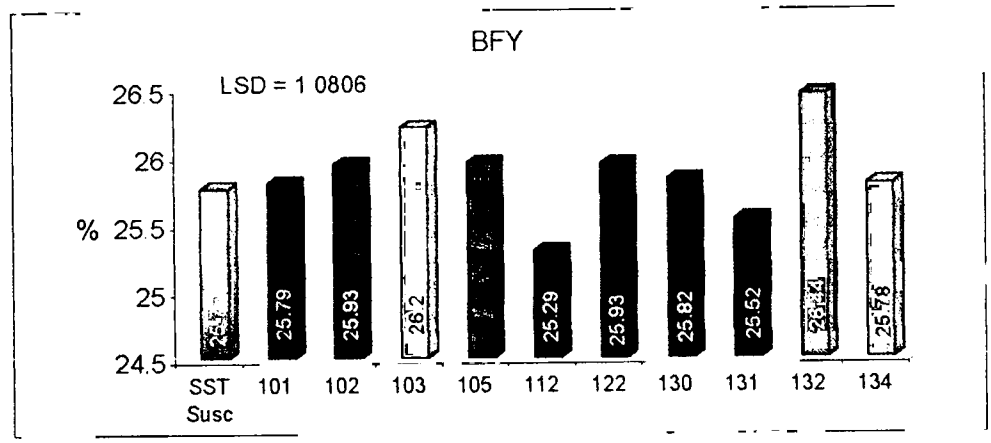

Figure 3.19 Breakflour yield of SST66 and its NIL's

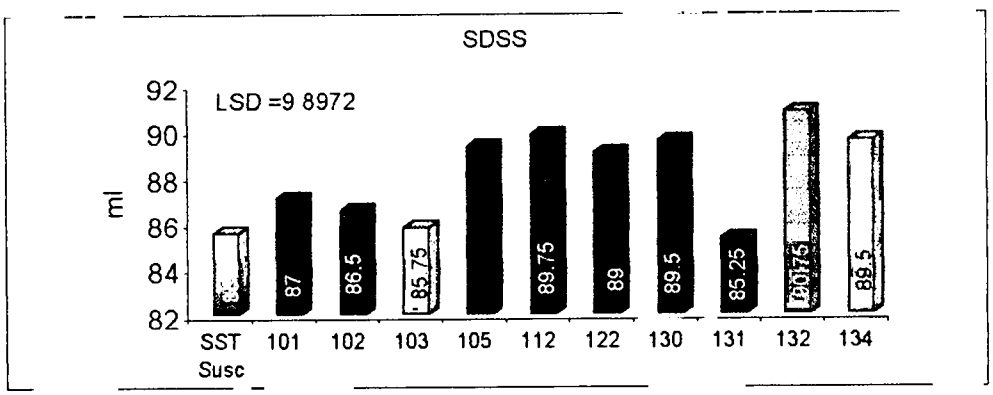

Figure 3.20 Sodium Dodecyl sulphate sedimentation of SST66 and its NIL's

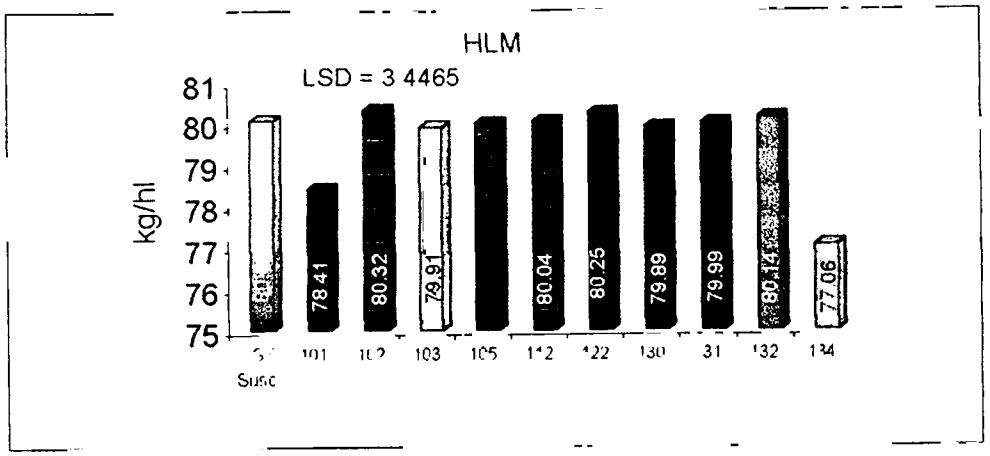

Figure 3.21 Hectolitre mass of SST66 and its NIL's

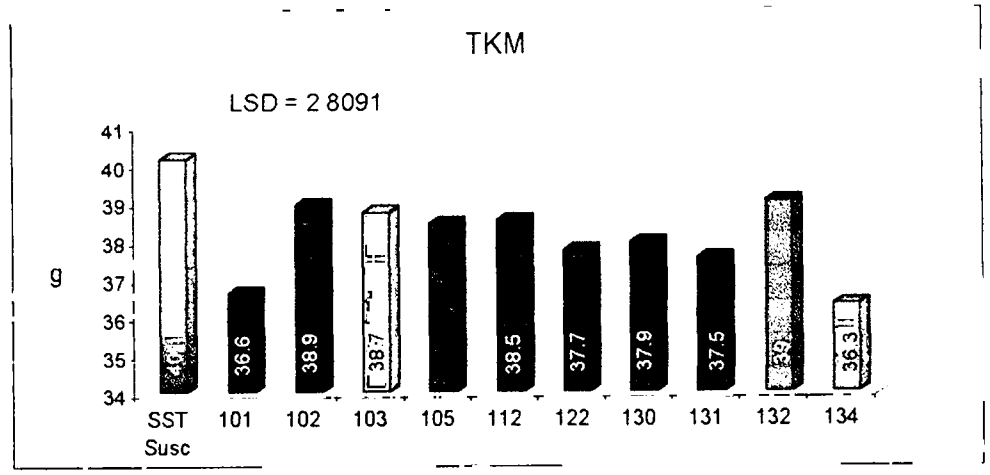

Figure 3.22 Thousand kernel mass of SST66 and its NIL's

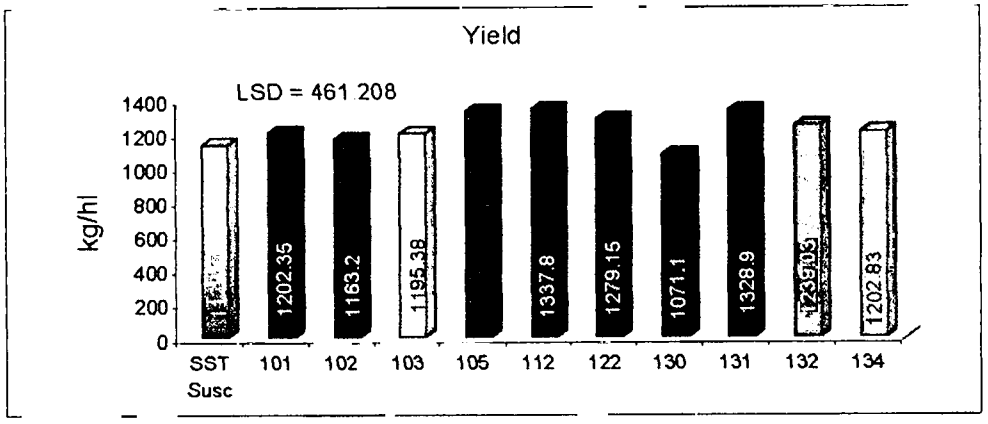

Figure 3.23 Yield per plot of SST66 and its NIL's

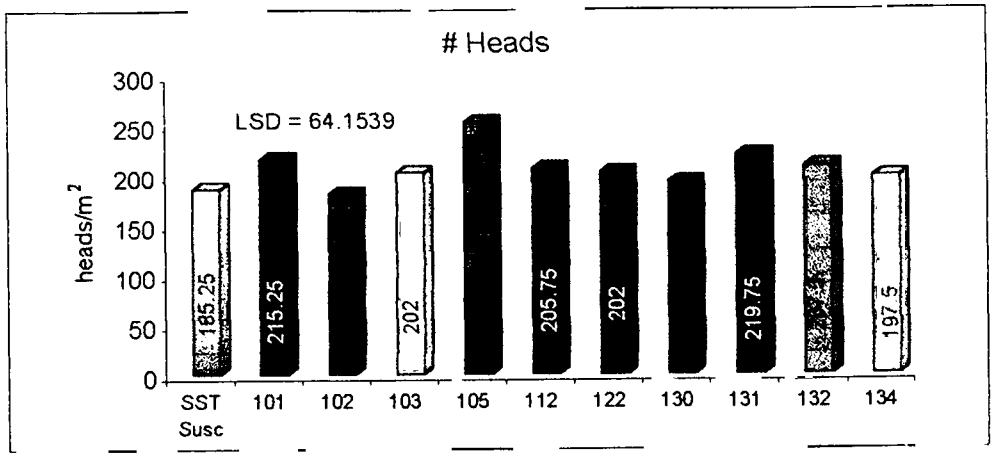

Figure 3.24 Number of heads per plot of SST66 and its NIL's

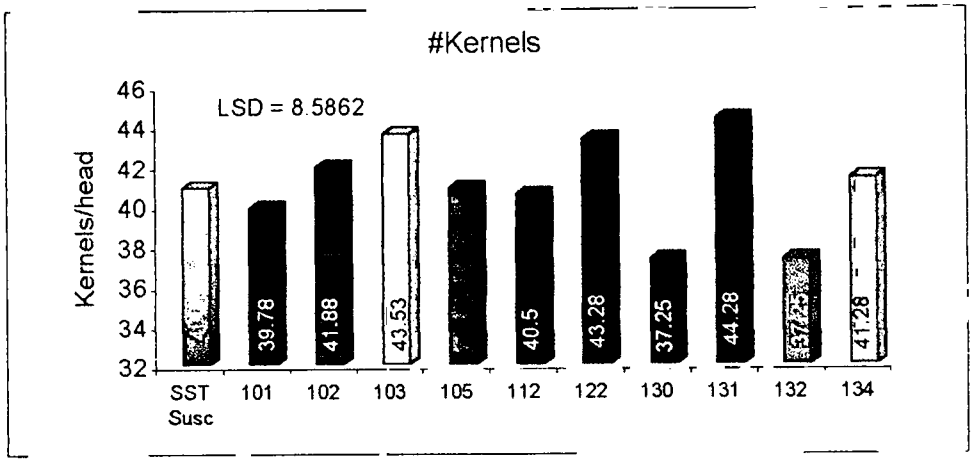

Figure 3.25 Number of kernels per head of SST66 and its NIL's

Rheological characteristics

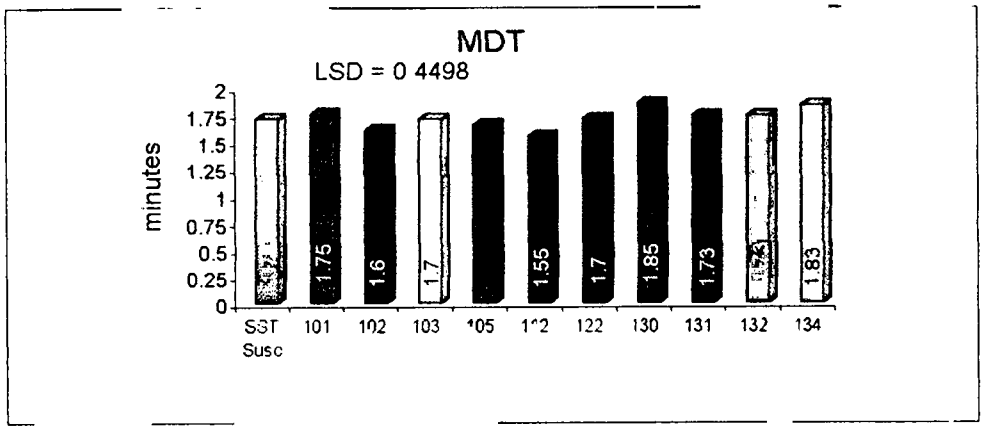

Figure 3.26. Mixograph development time of SST66 and its NIL's

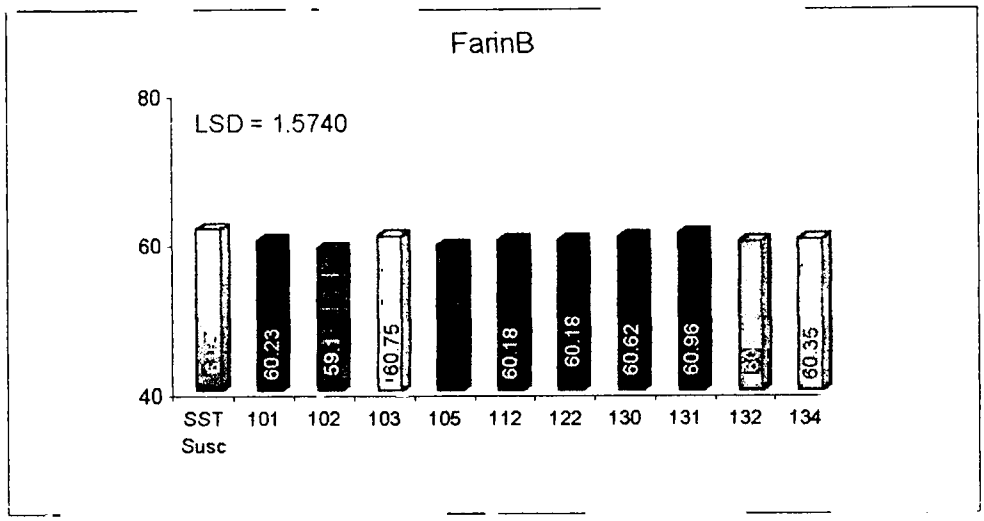

Figure 3.27 Farinograph water absorption of SST66 and its NIL's

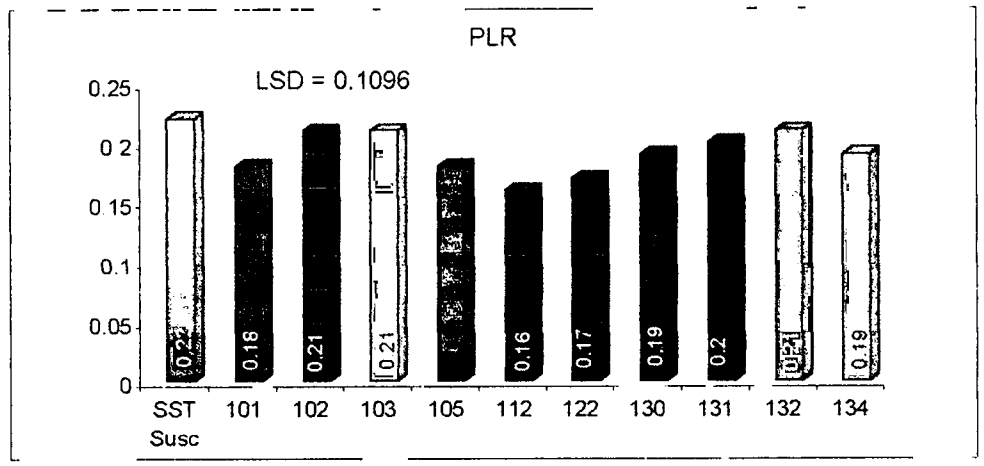

Figure 3.28 Alveograph P/L ratio of SST66 and its NIL's

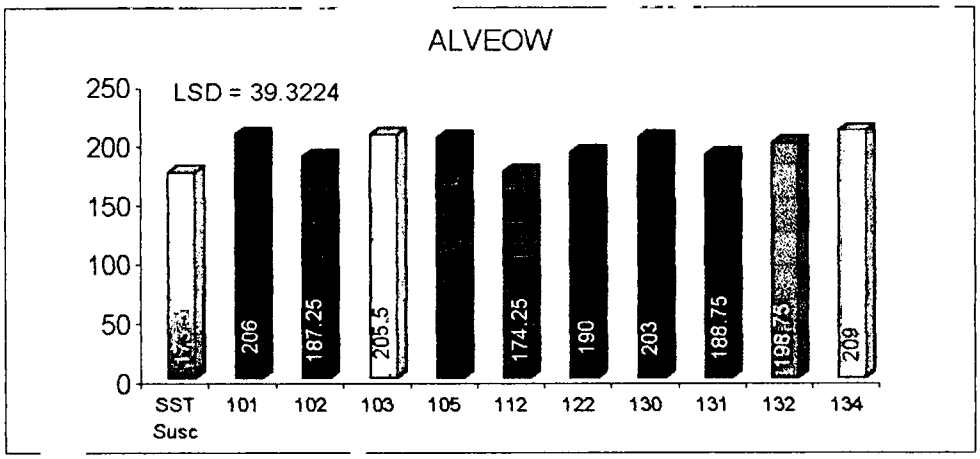

Figure 3.29 Alveograph strength of SST66 and its NIL's

Baking characteristics

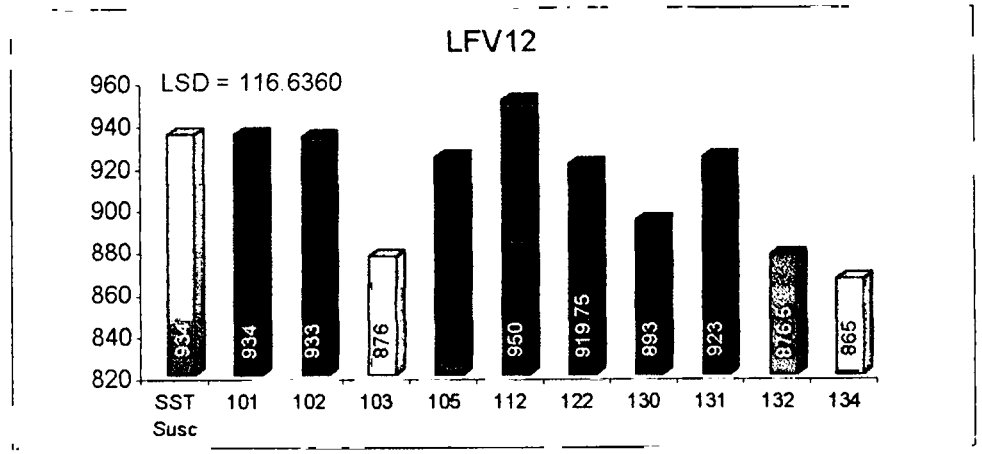

Figure 3.30 Loaf volume at 12% of SST66 and its NIL's

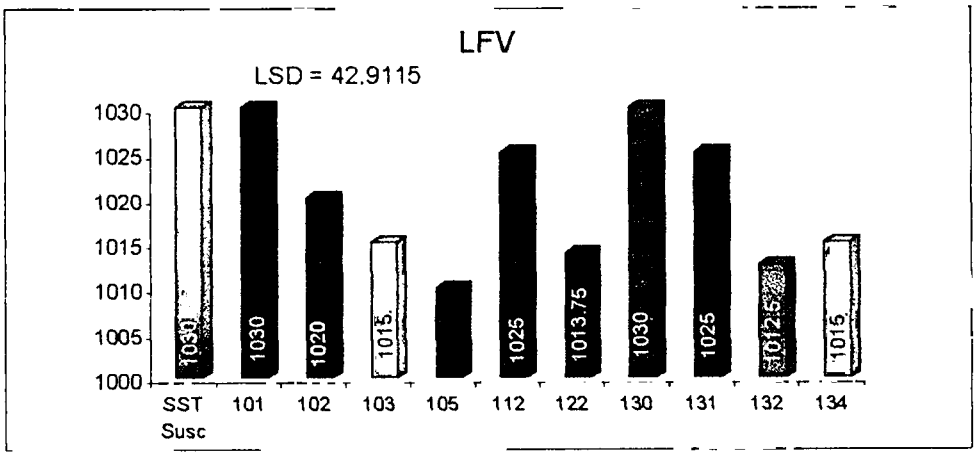

Figure 3.31 Loaf volume of SST66 and its NIL's

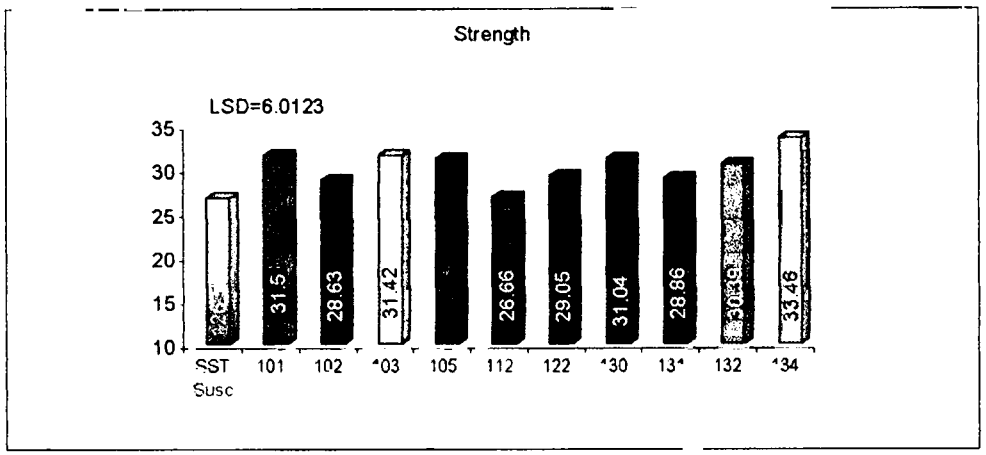

Figure 3.32 Strength of SST66 and its NIL's

3.4. Discussion

From the literature it is evident that eyespot has a major effect on the yield characteristics of wheat, under severe infections (Scott and Hollins, 1974). The effect of the resistance gene on the yield and baking quality components have not yet been studied.

All the progenies and parental lines showed averages higher than accepted, for flour protein content. The results show no scientific evidence that the eyespot resistant gene had any effect on the protein content. The same held true for flour extraction. A high extraction rate was obtained for all the individuals, with averages ranging from 70-74%. The presence of the gene, when compared to the susceptible parents had no influence on the extraction rates.

The presence of the gene had no significant influence on the breakflour yield. The resistant progeny gave better results than the susceptible parental lines for both Palmiet and SST66. It was evident from this test that, especially the Palmiet parent was harder than Karee. The literature suggests that harder wheat gives a higher SDS-sedimentation and higher flour yield value, the results obtained in this study disagrees with this finding, when the BFY is compared to the SDSS values (Eliasson and Larsson, 1993).

The results for the SDS-sedimentation tests and hectolitre mass was similar to that of the BFY, FPC and flour extraction, with the presence of the gene having no influence on these characteristics. The averages of the SDSS-tests for the different lines of both cultivars were higher than 70ml, indicating that the flours are of superior breadmaking quality. All the averages for the HLM were higher than the acceptable value of 76Kg.hl^{-1}.

The results for the yield characteristics were similar, with the presence of the gene having no apparent influence on these traits. The exception was the TKM

results which showed a higher value for the susceptible SST66 parent than for the resistant progeny. The SST66 progeny also had a somewhat lower TKM average than was expected. This, however may not be due to the presence of the gene. We would expect the Palmiet progeny's values also to be lower if this was caused by the *Pch1* gene.

Also for kernel number per head it was found that SST66 line 105 was the exception, as there was a significant difference between this resistant line and the susceptible parent. It is difficult to say if this is due to the presence of the gene or not. The fact that a significant difference is only visible in one line, might rather be ascribed to environmental influences, i.e. the position of this individual on the field, than to the presence of the gene.

The resistant isogenetic lines as well as the susceptible parental lines had very low mixograph mixing times. This indicates that suboptimal dough development took place. Finney and Shogren (1972) indicated that the MDT decreases as the flour protein content increases higher than 12%, as was the case here, although, the FPC is not that high in our case. Again, the presence of the gene had no detrimental effect on this characteristic.

Both the farinograph and alveograph results indicated that the presence of the resistance gene had no effect on these traits. The farinograph absorption for all the lines were at the optimum, but the P/L ratio was lower than the expected 0.8, which indicates that the dough is not that elastic (Walker and Hazelton, 1995). The low values obtained for the dough strength parameter, also proved this to be indicative of weak dough. It is obvious from the results that the dough from both the resistant and susceptible lines are weak, indicating that it is not because of the presence of the gene.

Both the susceptible parents had higher loaf volumes than most of the resistant progeny, with the exception of one progeny line. When the averages are

compared, it is clear that the presence or absence of the gene had no major impact on the results obtained in this study.

It is evident form the results of this research that the gene (*Pch1*) that causes eyespot resistance in wheat, had no effect on most of the important wheat quality characteristics. The presence of the gene will thus, ultimately

1. Protect the plant from the disease, and
2. Show no detrimental effect on any of the quality aspects of the wheat.

Chapter 4

Variability for storage proteins in two groups of near-isogenic lines

4.1. Introduction

The quality of good breadmaking wheat is highly dependable on its protein quality and quantity. Therefore, a lot research was done to study and identify the different proteins. The starchy endosperm of wheat consists of the storage proteins, structural proteins and enzymes. Storage proteins in wheats were divided into different groups on the basis of their solubility in different solvents (Osborne, 1907; Payne *et al.*, 1981; Eliasson *et al*, 1993). The two main groups forming the storage proteins are the glutenins (soluble in dilute acid or alkali) and the gliadins (soluble in aqueous ethanol).

The last 20 years has brought about an explosion of new knowledge on the biochemistry, genetics, functional properties and molecular biology of complex storage proteins. This modern era of wheat protein genetics was introduced with the advent of zone electrophoresis (Elton and Ewart, 1960; Woychick *et al*, 1961). It was now possible to separate the monomeric proteins in acid starch gels. Unfortunately the use of starch–gel electrophoresis was not without problems, it could not be used for separation of the glutenin fraction because the large molecular size made entry into the gel impossible. This problem was solved by the use of the SDS-PAGE method (Bietz and Wall, 1972). The use of these new methods made the genetic studies of these proteins possible.

Glutenins were further divided into two groups based on molecular weights i.e. High Molecular Weight Glutenin Subunits (HMW-GS) and Low Molecular Weight Glutenin Subunits (LMW-GS). These methods of protein separation were now used in genetic studies and it was possible to identify the genes involved in

controlling these subunits. Table 4.1 contains a list of the chromosomal location and the proteins coded for (Blackman and Payne, 1987).

Table 4.1 The chromosomal position of genes coding for the different storage (Blackman and Payne, 1987).

Gene locus	Chromosome Position	Arm	Storage Protein
Glu-A1	1A	Long (Close to centromere)	HMW-GS
Glu-B1	1B	Long (Close to centromere)	
Glu-D1	1D	Long (Close to centromere)	
Gli-A1	1A	Short (towards the end)	γ- and ω-gliadins and LMW-GS
Gli-B1	1B	Short (towards the end)	
Gli-D1	1D	Short (towards the end)	
Gli-A2	6A	Short (towards the end)	α- and β-gliadins
Gli-B2	6B	Short (towards the end)	
Gli-D2	6D	Short (towards the end)	

All of these studies were done in order to obtain a better understanding of the proteins so that their influence on the baking quality of wheat could be better explained.

Gliadins do not seem to have an important effect on baking performance (Reddy and Appels, 1990). When gliadins are interchanged between flours, the effect is minor compared to that of interchanged glutenins. Some groups of gliadins have been shown to be related to endosperm hardness, dough strength, Chopin values and Zeleny test. However, no correlation has been found between the above mentioned attributes and a single gliadin. Glutenin effects on these quality characteristics are much higher than that of gliadins, which makes the glutenin component more important to the breeder. Glutenins' major influences are on

the mixing properties of the dough, but they have also been implicated in the loaf volume.

The correlation between glutenin content and loaf volume is higher than the correlation between loaf volume and gliadin content. However it has been found that LMW-GS decreases mixing time as well as loaf volume. Whilst, HMW-GS increases both the loaf volume and the mixing time. This, then, explains the sensitivity of mixing behaviour to the gliadin/glutenin ratio. An increase in the gliadin fraction will cause the mixing time and the loaf volume to decrease, while an increase in the glutenin fraction causes both of these characters to increase. A longer mixing time is unwanted, thus by increasing the gliadin content the mixing time can be decreased. The loaf volume will remain unaffected, due to the broad range over which the glutenin/gliadin ratio works (Eliasson and Larsson, 1993,). LMW-GS are related to dough extensibilities and both the glutenin subunits contribute to dough resistance (Mita and Bohlin, 1983).

The aim of this research was to determine the genetic variability for storage proteins in two different sets of isogenetic lines. These protein fractions were not brought into relation with quality, due to a lack of time.

4.2. Materials and Methods

4.2.1. Isogenetic material

Seed material from near isogenic resistant Palmiet and SST66 lines were obtained from the SGI breeding program at Bethlehem. The resistant progeny lines were compared to their respective susceptible parent lines. The cultivar Chinese Spring was used as control in the LMW-GS and gliadin tests, while it was used together with Flamink and Betta for the HMW-GS tests.

4.2.2. High molecular weight glutenin subunits

4.2.2.1. Extraction of gliadins:

SDS-PAGE was used to determine the HMW glutenin subunits of each entry. The SDS-PAGE method of Singh *et al.* (1991) was adapted and used.

Seeds were crushed to a fine powder. Seventy percent ethanol (120µl) was added to each tube and incubated in the water bath at 60°C for an hour. Samples were vortexed every 20-40 min. The tubes were centrifuged for 2 min (high speed). The supernatant (75µl) was transferred to a new tube containing sample buffer (60mM Tris-HCl, 40g glycerol, 2g SDS and 0,02g bromophenol blue). Centrifuge as before. The samples were then ready to be loaded on a 10% polyacrylamide gel.

4.2.2.2. Extraction of glutenin:

The tubes containing the residue of seed were taken and the remainder of the supernatant removed. A millilitre of 50% 1-n-propanol was added to each tube and the seed material loosened. The tubes were incubated in the water bath at 60°C for 30 min and vortexed every 10 min. Centrifugation was done as before. The steps, mentioned above, were repeated. The seeds were washed with 0.5

ml 50% 1-n-Propanol, and all the supernatant removed. Eighty five microlitres of extraction buffer, containing 1.25% DTT (80mM Tris-HCl, 90ml 50% n-propanol and 0.1g to every 8ml extraction buffer, pH 8.0) was added. The seed material was loosened and incubated for a further hour. Another 85µl extraction buffer containing 16.8µl/ml vinyl pyridine (168µl vinyl pyridine to 10ml extraction buffer) was added, and the samples were placed back in the waterbath for another hour. Centrifugation for 2 min at high speed was done and the supernatant transferred to new tubes containing 110µl sample buffer consisting of 80 mM Tris-HCl (pH8), 40g glycerol, 2g SDS and 0.02g Bromophenol blue. Samples were vortexed and incubated for 15 min at 60°C, centrifuged again and loaded, 65µl (if extracted from half seeds) or 45 µl (if whole seeds were used) on a 10% polyacrylamide gel.

4.2.3. Gel electrophoresis

A two-part polyacrylamide (10% uniformity) gel was run at 72 mA at 15°C for 3 hours (adopted from Singh *et al.*, 1991). The first part to be poured is the separating (separating buffer, separating acrylamide, water, APS, and temed) gel. After it has polymerised (approximately 1 hour) the stacking gel (stacking buffer, stacking acrylamide, water, APS and temed) is poured on top. The gel was fixed in fixing solution (400ml methanol, 100ml glacial acetic acid, 500ml H_2O) and then stained (150g trichloro-acetic acid, 0.1g coomassie blue and 50 ml methanol).

Gel analyses were done with the help of the " Molecular Analyst Fingerprinting" software of Biorad. The gel was scanned or photographed by the UV-gel camera of the gel Doc 1 000. These gel images were first converted, then normalised and finally analysed by the programme. The clearest Chinese Spring pattern was used as the standard reference and the other Chinese Spring reference patterns were aligned to the standard reference. The analyses were done by comparing the densitometric curves obtained for each sample. From the curve it

is possible to determine the migration distances of the replications of the different lines. The peak heights are of discriminative importance since only peaks with intensities higher than 15% were accepted. Replications were also compared for their repeatability. Only bands with a repeatability of higher than 50% were accepted as being representative of the line. The averages of the migration distances for the six replications were calculated and used to compare and indicate the specific patterns.

4.2.4. Nomenclature

The nomenclature system of Gupta and Shepherd (1988) was used for the LMW-GS, and Konarev *et al.* (1979) system was used for the gliadins, as explained in the sections 2.6.1.2 and 2.6.2 in Chapter 2.

4.3. Results

4.3.1. HMW-GS

Table 4.2 contains a summary of the HMW-GS banding patterns observed, of all the progeny and parent lines, as obtained from a 10% SDS-PAGE gel. Six replications were done and only the patterns that were present at least 50% of the time are listed.

The banding combination of the Palmiet susceptible parent line was, 2*, 5 +10, 13 + 16. The Palmiet progeny all displayed the same banding pattern, 0, 5 +10, and 13 + 16. Lines 208 and 213 were the exception, with an added band, 1.

Table 4.2 the HMW-GS banding patterns observed for the different SST 66 and Palmiet progeny lines and their parents.

Lines	HMW-Glutenin band patterns						
	A Genome			B Genome		D Genome	
	0	1	2*	2+12	5+10	7+8	13+16
SST66 101		✓		✓		✓	
SST66 102		✓		✓		✓	
SST66 103		✓		✓		✓	
SST66 105		✓		✓		✓	
SST66 112		✓		✓		✓	
SST66 122		✓		✓		✓	
SST66 130		✓		✓	✓	✓	
SST66 131		✓		✓	✓	✓	
SST66 132		✓		✓		✓	
SST66 134		✓		✓		✓	
SST 66 parent	✓			✓		✓	
Palmiet 202	✓				✓		✓
Palmiet 203	✓				✓		✓
Palmiet 207	✓				✓		✓
Palmiet 208	✓	✓			✓		✓
Palmiet 211	✓				✓		✓
Palmiet 212	✓				✓		✓
Palmiet 213	✓	✓			✓		✓
Palmiet parent			✓		✓		✓

The banding combination for SST 66 susceptible parent line, 1, 7 + 8, 2 + 12, differed slightly from that which were observed. The parent line had a 0 instead of a 1. The progeny all contained the banding combination, 1, 7 + 8, and 2 +12. Again two lines, 130 and 131, appear to be the exception with having 5 + 10 added to their combination.

4.3.2. LMW-GS

The following tables contain the data obtained for the LMW-GS of the different lines of the two cultivars. The first six columns contain the migration distances for the six replications, the next column contains the accepted averages and the last, the repeatability of the result.

Table 4.3 Migration distances of the LMW glutenin subunits of SST 66 line 101.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Average	Repeat.
5	7	3				5.00	50.0
19	17	22				19.33	50.0
		25		29	30	28.00	50.0
37		40	41	42		40.00	66.6
56	51	54	52		52	53.00	83.3
67	67		67	67		67.00	66.6
71		72	72		72	71.75	66.6
	76	80	81	78	80	79.00	83.3
87			91	87	90	88.75	66.6
	97	96	95	98	96	96.40	83.3
109		106	107		102	106.00	66.6
	111			110	114	111.67	50.0
123	126	122	124	123		123.60	83.3
133	132	138		133	138	168.50	66.6
163		165		160	162	162.50	66.6
175			175	180	183	178.25	66.6

Table 4.4 Migration distances of the LMW glutenin subunits of SST 66 line 102.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Average	Repeat
8	7	7	13		14	9.800	83.33
29		21			19	23.000	50.00
30			36	32	32	32.500	66.66
45	44	48	42	40	42	43.500	100.00
57	53		56	52	53	54.200	83.33
			64	64	62	63.333	50.00
82	80	77	83			80.500	66.66
	86	87		86	87	86.500	66.66
			91	93	93	92.333	50.00
			98	100	100	99.250	66.66
	99		104	104	104	104.000	50.00
112		119	116	119	120	117.167	100.00
128	117	135		131	128	130.600	83.33
146	131	144	145	145	147	148.167	100.00
162	162	162		166		163.000	66.66
	188		186	189	189	188.000	66.66

Table 4.5 Migration distances of the LMW glutenin subunits of SST 66 line 103.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Average	Repeat
7	6	5				6.000	50.00
10	11	10				10.333	50.00
		20	21	20	20	20.250	66.66
31		31		28	29	29.750	66.66
34		36	33	35	35	34.600	83.33
			44	42	41	42.333	50.00
48	51	51	52	51	50	50.500	100.00
		58	59	58	55	57.500	66.66
60	62	61	63	62	61	61.500	100.00
	68	66	67	67	68	67.200	83.33
80	80	80	78	78		79.200	83.33
88	86		84	82	81	84.200	83.33
92	89	92	93	92	90	91.333	100.00
	95	97		95	98	96.250	66.66
106			105	104	103	104.500	66.66
		113	112	112		112.333	50.00
121	124	126	121	121	122	122.500	100.00
139	138		137		134	137.000	66.66
		140		142	142	141.333	50.00
147	148	150	148	148	148	148.167	100.00
153	155	155	153			154.000	66.66
163	165	168		167	160	164.600	83.33
	171	174				172.500	33.33
	182	182				182.000	33.33
		192	193	193	193	192.750	66.66

Table 4.6 Migration distances of the LMW glutenin subunits of SST 66 line 105.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Average	Repeat
8	6	5		8		6.75	66.66
11	11	14	16	19		14.2	83.33
27	28	27	27		27	27.2	83.33
32	31	30	32	30		31	83.33
44	46	45	44		44	44.6	83.33
52		56	54	50	57	53.8	83.33
62	60		63	64		62.25	66.66
74		74	72	74	75	73.8	83.33
77	76		76	77		76.5	66.66
84	83	80	79		82	81.6	83.33
	86	84	89	88		86.75	66.66
91	91		96	95	93	93.2	83.33
104			100	100	100	101	66.66
108	112		110		105	108.75	66.66
118	119	117	114		111	115.8	83.33
			124	122	125	123.667	50.00
133	132	130	130	131		131.2	83.33
136		139	136	134	137	136.4	83.33
	142	143	140		140	141.25	66.66
144	147	145	145			145.25	66.66
151	149		150	154		151	66.66
159	156	155			155	156.25	66.66
165			161		161	162.333	50.00
			171		169	170	33.33
	185	184				184.5	33.33
	187	189				188	33.33
		200	198	198	200	199	66.66

Table 4.7 Migration distances of the LMW glutenin subunits of SST 66 line 112.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Average	Repeat.
11	16	16				16	50.00
	21	21	20	21	22	21.000	83.33
	29	32		28	30	29.750	66.66
38	37					37.500	33.33
	41	41	41	41	42	41.200	83.33
47	49			46	49	47.750	66.66
	51	52		50		51.000	50.00
67	69	66				67.333	50.00
72	72	74	70	70	71	71.500	100.00
	77	78	78	79		78.000	66.66
82	80	81			81	81.000	66.66
93	91		91	91	93	91.800	83.33
	97	99	99	100	102	99.400	83.33
	106	106	108	106	108	106.800	83.33
112	114	114	111	111	114	112.667	100.00
129	126	126	125	123	128	127.167	100.00
133	132				134	134.000	50.00
139	136	135	139			138.250	66.66
141	141	140		142	146	143.000	83.33
149	149	148	149		149	149.800	83.33
	158	157	155	158	158	158.200	83.33
	170	170		170	166	170.000	66.66
177	177	173				176.667	50.00
179	182	180	182	183		182.200	83.33
189	189	188	187	186		188.800	83.33
200	200	200	200	200		201.000	83.33

Table 4.8 Migration distances of the LMW glutenin subunits of SST 66 line 122.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Average	Repeat
11		8	9			10.333	50.00
20			22	17	17	20.000	66.66
	28			28		28.000	33.33
39		33			32	34.667	50.00
	44	45	43	40	44	44.200	83.33
					48	48.000	16.66
62	66		59	66		64.250	66.66
67		70	69		68	69.500	66.66
	77	81		76	79	79.250	66.66
91	91		88	88	94	91.400	83.33
108	106	99	102	102	108	105.167	100.00
114		113				113.500	33.33
121	119		118	118		120.000	66.66
124	124					124.000	33.33
126	128	130	127		126	128.400	83.33
134	132	133	133			134.000	66.66
138	138	138		136	139	138.800	83.33
	153	149		143	149	149.500	66.66
169		162	168		169	168.000	66.66
175	183					179.000	33.33
189	192	193				191.333	50.00

Table 4.9 Migration distances of the LMW glutenin subunits of SST 66 line 130.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Average	Repeat
3	3	3				3.000	50.00
	7					8.000	16.66
	14		19	15	12	15.000	66.66
25	25	25		26	24	25.000	83.33
36	37	38	31	40		37.400	83.33
70			67	67	65	67.250	66.66
86	75	78	81	81	81	81.333	100.00
	90	90	97	97	98	95.400	83.33
106	108	108				108.333	50.00
119	121		112	114	120	118.200	83.33
131	132	126	131	132	134	131.000	100.00
136	138	139	140	140		232.000	50.00
151	152	156	153	155	150	153.833	100.00
162	162		159	159	161	161.600	83.33
		167	171	175		172.000	50.00
	183				190	186.500	33.33

Table 4.10 Migration distances of the LMW glutenin subunits of SST 66 line 131.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Average	Repeat
6	6	9				7	50.00
	12		11	12	11	12.5	66.66
18	16	18				17.3333	50.00
26		25	24	22	21	24.6	83.33
38	37	37				37.3333	50.00
62	64	65	63	62	62	64	100.00
77	76	76		80		78.25	66.66
			83	83	83	84	50.00
91	92	92	88	88	88	89.8333	100.00
			101	105	107	105.333	50.00
114	115	114				114.333	50.00
126	126	125	122	122	127	125.667	100.00
141	139	139	137	139	142	140.5	100.00
154	152	152	152	154	157	154.5	100.00
168	166	164	161	163	166	165.667	100.00
173						173	16.66
200	200	200	200	200	200	200	100.00

Table 4.11 Migration distances of the LMW glutenin subunits of SST 66 line 132.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Average	Repeat
8	7	10		11	11	11.750	66.66
15	12		13	16	16	15.400	83.33
20	26	20	25			22.750	66.66
33		34		30	33	32.500	66.66
41	41		39	42		41.750	66.66
48		48			49	48.333	50.00
63	70		66			67.333	50.00
74	73	76			77	76.000	66.66
81	82					82.500	33.33
90	93	97	96	99	97	96.333	100.00
113			114	117	117	115.250	66.66
124	129	129	131	132	131	130.333	100.00
136	136		133	134	134	135.600	83.33
146	140	141	146	147	146	145.333	100.00
	154	156				155.000	33.33
161	163	166	160	161	161	163.000	100.00
175			169	169	170	170.750	66.66
185	182	193	182	183	183	184.667	100.00
175	183					179.000	33.33
189	192	193				191.333	50.00

Table 4.12 Migration distances of the LMW glutenin subunits of SST 66 line 132.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Average	Repeat
4		4			6	7.000	33.33
	18	17	18		15	18.000	66.66
25		20		20		21.667	50.00
37	39	31	34	36	39	37.000	100.00
48		41	48	50		62.333	50.00
		55			54	55.500	33.33
		58	60	60	59	59.250	66.66
72	72	72	77	78	78	75.833	100.00
90	90	90	95	94	94	93.167	100.00
104	104	104				104.000	50.00
			114	114	114	115.000	50.00
121		121		125	124	122.750	66.66
	127	129	128	126		128.500	66.66
136	138	139	130			136.750	66.66
146			145	141	141	144.250	66.66
	150	150	158	155	152	154.000	83.33
164	162	163	168	165	163	164.167	100.00
177	178	180	182	181	180	180.667	100.00
186		187				187.500	33.33
	197	197	200	200	193	198.400	83.33

Table 4.13 Migration distances of the LMW glutenin subunits of SST 66 parent line.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Average	Repeat.
6	6		6		6	6.00	66.6
27	26	26	27	28	28	27.00	100.0
42	43	44	44	43		43.20	83.3
58	59	60	62		61	60.00	83.3
72	75	76			74	74.25	66.6
85					85	85.00	33.3
	98	98	98	98	98	98.00	83.3
110	115			114	115	113.50	66.6
124	124	124	125	126	125	124.67	100.0
150	147	146		146		147.25	66.6
159			159	159	159	159.00	66.6
168		168	168	168	168	168.00	83.3
183		183	183	183		183.00	66.6
194	195	197		195		195.25	66.6

Table 4. 14 Migration distances of the LMW glutenin subunits of Palmiet line 202.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Average	Repeat
7		5				6	33.33
32	33		25	25	29	29.8	83.33
40			37	37	38	38	66.66
47	49		47	48	50	49.2	83.33
	60	62	60	55	58	59	83.33
69	71		77	77	75	73.8	83.33
86	87	87	84		87	87.2	83.33
		93	91	91		92.6667	50.00
112	115	115	111	111		113.8	83.33
118	121	120				119.667	50.00
126	126	130				128.333	50.00
134	135	137	134	136	136	135.333	100.00
142						143	16.66
155		154				155.5	33.33
		170				171	16.66
		189	188	188	189	189.5	66.66

Table 4.15 Migration distances of the LMW glutenin subunits of Palmiet line 203.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Average	Repeat
11		10	11	9	5	9.2	83.33
	16	13	14	13	14	15	83.33
26	28	24	22	22		24.4	83.33
				37	37	38	33.33
			50	45	50	48.3333	50.00
			57	57		58	33.33
60	62	64	64	64	61	62.5	100.00
65	64		67	69	67	67.4	83.33
				73	72	73.5	33.33
	80	80	81	78	78	80.4	83.33
88	89	88	89	87	91	88.6667	100.00
			96	95	98	97.3333	50.00
			102	101	105	102.667	50.00
114	113	115			113	114.75	66.66
	119	121	121	126	117	121.8	83.33
	130	130		131	130	131.25	66.66
135	136	137	139	139	137	138.167	100.00
		143	144	142		143	50.00
152	155		150	155		153	66.66
			163	163	162	162.667	50.00
	171		169	170	171	170.25	66.66
		189	189	188		188.667	50.00
		195	199	199	200	199.25	66.66

Table 4.16 Migration distances of the LMW glutenin subunits of Palmiet line 207.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Average	Repeat
13	17	17	15	18	18	16.3333	100.00
27			25		25	26.6667	50.00
	32	32			35	33	50.00
40	47	47				45.6667	50.00
			53	55	55	54.3333	50.00
			62	64	64	64.3333	50.00
70	70	70	69	71	69	69.8333	100.00
			75	77	75	76.6667	50.00
85	88	86	79	80	79	83.8333	100.00
			94	99	97	96.6667	50.00
			102	106	105	105.333	50.00
115	114	113	110	114	115	113.5	100.00
129	129	129	127	122	122	127.333	100.00
140	138	138	139	140		140	83.33
144	147	146			146	146.75	66.66
152		154	155	150		153.75	66.66
			164		164	164	33.33
177	182	180			180	180.75	66.66
	189	189	193	193	191	192	83.33
			198	198		199	33.33
200	200		200	200	200	201	83.33

Table 4.17 Migration distances of the LMW glutenin subunits of Palmiet line 208.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Average	Repeat
5	4	3				4	50.00
8	8	8		8		9	66.66
14	16			13		14.3333	50.00
24	24			23		24.6667	50.00
35	34	31	38	39	39	36	100.00
45	45	44				45.6667	50.00
60	61			61	61	60.75	66.66
69			65	67	68	68.25	66.66
	70	70	70	72	70	71.4	83.33
			81	82	83	83	50.00
92	96	97	94	98	98	95.8333	100.00
			100	103	104	103.333	50.00
112	112	113	119			114	66.66
122	124	124		122	121	123.6	83.33
133	132					133.5	33.33
135	138	136				137.333	50.00
145			146	145	146	146.5	66.66
	150	150		149	149	149.5	66.66
155	160	160				159.333	50.00
171			174	178	179	175.5	66.66
			200	195	195	196.667	50.00

Table 4.18 Migration distances of the LMW glutenin subunits of Palmiet line 211.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Average	Repeat
	9	5	8	8		7.5	66.66
16	18				17	18	50.00
25			24			24.5	33.33
38	34			36	34	36.5	66.66
45		50	42	49	48	46.8	83.33
	57		60	56	59	59	66.66
68			66	65		66.3333	50.00
95	90	90	95	93	90	93.1667	100.00
110	105	108	105			108	66.66
	121	120	121	120		120.5	66.66
125		128	124		124	126.25	66.66
132	134	133				133	50.00
138	136	135		139		138	66.66
	142	140			141	142	50.00
146			147	146		147.333	50.00
	152		150			152	33.33
		169				169	16.66
177	177	175	178	177	174	177.333	100.00
186	184			187	184	185.25	66.66
	189		193	192	192	191.5	66.66

Table 4.19 Migration distances of the LMW glutenin subunits of Palmiet line 212.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Average	Repeat
4						4	16.66
	10		13			12.5	33.33
19	22	20	19	19		19.8	83.33
		36	32	39	35	35.5	66.66
40	42	42				42.3333	50.00
50			46	48	49	48.25	66.66
		64	64	67	62	65.25	66.66
79			70	76	74	75.75	66.66
		90	94	91	90	91.25	66.66
103	105	101				104	50.00
			116	112	112	113.333	50.00
121	121	119		119	121	121.2	83.33
127	128	127	129	126	129	128.667	100.00
132	131	132	132			132.75	66.66
141	143	141	143		143	143.2	83.33
153	156		155		155	154.75	66.66
169	172	174			173	173	66.66
	179	179	175	175		177	66.66

Table 4.20 Migration distances of the LMW glutenin subunits of Palmiet line 213.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Average	Repeat
	33		33	33	33	33	66.66
42	43	40				42.6667	50.00
		47	47	47	46	46.75	66.66
70	72	73	72	71	71	72.5	100.00
90	90	90	88	88	88	89	100.00
		99	96	95	98	98	66.66
		107	109	109	108	108.25	66.66
119	119	117		117	115	118.4	83.33
124			120		123	123.333	50.00
128	128	127	129	128		129	83.33
134	134	133	130		134	133	83.33
137	138	139	138	137		138.8	83.33
147	148	146	150	149	145	147.5	100.00
161	160		167	167	163	164.6	83.33
177	179	179				179.333	50.00
186	186	186			188	187.5	66.66

Table 4.21 Migration distances of the LMW glutenin subunits of Palmiet parent line.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Average	Repeat.
4	4	4	5	3	6	4.33	100.0
14	11	15	10			12.50	66.6
28	27	28		29	30	28.40	83.3
37	36		36	39		37.00	66.6
51	51	52	52		52	51.60	83.3
60	59	60			61	60.00	66.6
	77	77	77	77	77	77.00	83.3
88	87	87	87	87	88	87.33	100.0
97	96	98		98	98	97.40	83.3
102	103	103	102			102.50	66.6
116	116	116	116	116	116	116.00	100.0
140	139	140	140		140	139.80	83.3
159		159	159	159		159.00	66.6
169	168	170		170	171	169.60	83.3
173		173	173	172	173	172.80	83.3
	200		200	200	200	800.00	16.6

Table 4.22 Summary of the lines tested and their different LMW-subunit combinations. Using the nomenclature system of Gupta and Shepherd (1988).

Cultivar	Glu-A3	Glu-B3	Glu-D3
SST66 101	f	a; c; e; g	a; c; d; e
SST66 102	c; f	a; e	a; c
SST66 103	a; c; f	a; b; e; f	b; c
SST66 105	a; f	a; b; e; f	a; b; d; e
SST66 112	a; b	a; b; e; f	a; b; c; d, e
SST66 122	c; f	e	b; c
SST66 130	c	f	b; c; e
SST66 131	a; c; f	f	a; c; e
SST66 132	a; b; c	d	a; b; c; d; e
SST66 134	a; b; c	f	a; b; c; d; e
SST66	a; b; f	b; e	a; b; c; d; e
Palmiet 202	a; f	a; b; e	a; b; c; d; e
Palmiet 203	b; c; d; f	a; d;	c
Palmiet 207	a; b; c; f	e; f	b
Palmiet 208	f	e	b
Palmiet 211	a	a; f	d
Palmiet 212	b; f	e	a; b; c; d; e
Palmiet 213	a; f	c	a; d
Palmiet	b; d; f	a; b; c; e; f	a; b; c; e

Chromosome 1A, group1

All the SST 66 progenies contained the a combination, except lines 101, 102, 122 and 130. Line 130 only contained the c combination, only lines 105, 112 and the parent did not have this combination. Lines 112, 132 and 134 had the b combination common to the parent line. The susceptable parent is the only line containing the d combination. Another combination the isogenetic lines shared with the parent is the f combination.

The Palmiet isogenetic lines carried the f combination except for line 211, which only had the a combination. Lines 202, 207 and 213 also had this combination. Lines 203, 207, 212 and the susceptible parent also had the b combination. Only the parent line and line 203 had the d combination.

Chromosome 1B, group 2

Most of the resistant SST 66 isogenetic lines had the e combination. Lines 122, 131 and 134 only the f combination, while lines 122 and 132 had combinations e and d respectively. Line 102 had both combinations a and e. Line 101 is the only line with combinations c and g. Whereas the parent , line 103 and line 105 are the only ones which had combination b.

Four of the Palmiet progeny lines (202, 207, 208 and 212) and the parent line had the e combination. Line 207 also had the f combination, as did line 211. Line 211 and lines 202, 207 and the parent line had combination a. the parent line and line 213 are the only lines with the c combination. The parent line also had combination b as did line 202. Only line 203 had the d combination.

Chromosome 1D, group 3

All the SST 66 lines, including the parent line had the c combination, with the exception of line 105. The parent line and most of the progeny also had the b combination, only lines 101, 102 and 131 did not have this combination. Lines 103, 102, 105, 112, 131, 132, 134, and the parent line had the a combination. Most of the lines, with the exception of lines 102, 103, and 122, had the e combination. Only lines 101, 105, 112, 132, 134 and the parent line had the d combination. Four lines (112, 132, 134 and SST 66 parent) had all the possible combinations.

All the Palmiet lines were found to have the b combination, except lines 203, 211, and 213. Lines 202 and 212 had all the possible combinations. The Palmiet parent line had all the combinations except for combination d. Line 211 had only combination d, whereas line 203 had only combination c. Line 213 had both the a and d combinations.

4.3.3. Gliadins

The data of each line of both cultivars are summarised in the following tables. The first six columns, as with the LMW GS, contain the migration distances. The next column contains the averaged values accepted as the peak positions and the last column shows the repeatability of the result.

Table 4.23 Migration distances of the gliadin subunits of SST 66 line 101.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Average	Repeat.
			4	4	4	4.00	50.0
			9	10	10	9.67	50.0
15	15	16	16	16	16	15.67	100.0
		22	20			21.00	33.3
		24				24.00	16.6
27	27	28	26	27	27	27.00	100.0
31	31	31	31	30	30	30.67	100.0
36	35	36		34	34	35.00	83.3
40	40	41				40.33	50.0
43		44				43.50	33.3
49	50	50	50	50	50	49.83	100.0
	54	54				54.00	33.3
		58			59	58.50	33.3
64	64	68	62	65	67	65.00	100.0
73	76	75	72	72	73	73.50	100.0
79		78	78	77	78	78.00	83.3
	80	81	82	83	82	81.60	83.3
86	85		85	85	84	85.00	83.3
	88	89		88		88.33	50.0
90	90		90	91	90	90.20	83.3
			92	94	94	93.33	50.0
98	98	98	98	98	99	98.17	100.0
			101	102		101.50	33.3
105	104	105	104	106	104	104.67	100.0
			107	109	107	107.67	50.0
110	112	112	111		110	111.00	83.3
117	117	116	113	116	113	115.33	100.0
			117		118	117.50	33.3
	122	122			124	184.00	33.3
126	127	127		126	128	158.50	66.6
132		133				132.50	33.3
137	137	136		136	139	137.00	83.3
140	142					141.00	33.3
147				147	148	147.33	50.0
	154			152	152	152.67	50.0
156	158	160	158		158	158.00	83.3
161		161				161.00	33.3
165	165	165	165		163	164.60	83.3
			167		167	167.00	33.3
			169	170		169.50	33.3
172	172	172		173	172	172.20	83.3
178	176		177			177.00	50.0
	183	182	183	183	184	183.00	83.3
188	186	186	188	188	188	187.33	100.0
191	191		195	197	198	194.40	83.3
200	200	200				200.00	50.0

Table 4.24 Migration distances of the gliadin subunits of SST 66 line 102.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Average	Repeat.
0	0	0	0	0	4	4.00	16.6
0	8	0	7	10	9	11.33	50.0
16	14	14	12			14.00	66.6
0	18	0	15	16	15	12.80	83.3
20	20	0	22	21	22	21.00	83.3
27	27	26	27	27	27	26.83	100.0
31	0	30	30	31	30	30.40	83.3
36	34	34	34	35	34	34.50	100.0
0	0	0	44	42	44	43.33	50.0
51	53	49	49	50	49	50.17	100.0
0	0	0	57	58	57	57.33	50.0
62	61	60	62	63	63	61.83	100.0
65	66	65	66	65	65	65.33	100.0
0	70	0	72	72	70	56.80	83.3
79	77	77	77	77	77	77.33	100.0
84	85	82	83	81	82	82.83	100.0
87	87	88	89	89	89	88.17	100.0
92	0	0	93	92	93	92.50	66.6
98	96	96	96	95	97	96.33	100.0
104	102	102	100	100	99	101.17	100.0
0	104	0	109	108	108	107.25	66.6
0	112	115	112	113	110	112.40	83.3
119	119	0	117	118	118	118.20	83.3
127	125	128	126	126	126	126.33	100.0
136	136	0	135	135	135	135.40	83.3
0	0	0	142	143	141	142.00	50.0
0	146	0	146	147	147	146.50	66.6
0	152	0	153	153	155	153.25	66.6
0	161	0	165	166	166	164.50	66.6
0	172	0	172	173	172	172.25	66.6
0	0	0	178	178	178	178.00	50.0
0	182	182	181	182	181	181.60	83.3
186	187	185	186	186	186	186.00	100.0
	0	188	190	190	0	189.33	50.0
193	0	193	193	193	193	193.00	83.3
200	200	200	198	197	196	198.50	100.0

Table 4.25 Migration distances of the gliadin subunits of SST 66 line 103.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Average	Repeat.
			9	9	9	9.00	50.0
14	14	14	15	14	13	14.00	100.0
			20	20	19	19.67	50.0
26	26	27	27	26	25	26.17	100.0
29			30	29	29	29.25	66.6
34	32	34	34	33	33	33.33	100.0
48	47	48	48	48	48	47.83	100.0
			58	58	58	58.00	50.0
63	62	62	62	61	60	61.67	100.0
			65	64	64	64.33	50.0
			70	68	69	69.00	50.0
76	76	78	76	75	75	76.00	100.0
81			81	82	80	81.00	66.6
84	84	84	84		82	83.60	83.3
88	88	90	89	88	86	88.17	100.0
94	94		93	92	92	93.00	83.3
102	99	102	103	100	102	101.33	100.0
			107	106	105	106.00	50.0
115	113		117	114	115	114.80	83.3
122				123	122	122.33	50.0
128	128		126	126	127	127.00	83.3
138		139		137	139	138.25	66.6
			151	151	151	151.00	50.0
175			171	170	170	171.50	66.6
178				178		178.00	33.3
180			184	184	184	122.00	100.0
192		197	195	194	194	194.40	83.3

Table 4.26 Migration distances of the gliadin subunits of SST 66 line 105.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Average	Repeat.
		3	9	10	4	6.50	66.6
15	15	15	14	14	14	14.50	100.0
			20	20	21	20.33	50.0
27	27	27	26	26	27	26.67	100.0
			30	29	30	29.67	50.0
34	34	34	34	33	35	34.00	100.0
			39	39	39	39.00	50.0
			43		44	43.50	33.3
49	48	49	48	48	49	48.50	100.0
				58	58	58.00	33.3
60	59	60	61		61	75.25	66.6
64	64	64	64		64	64.00	83.3
72	71	70	67	67	68	69.17	100.0
75	77	77	75	77	76	76.17	100.0
84	84	83	83	84	85	83.83	100.0
	90	88	89	88	89	88.80	83.3
	96	96	98	99	99	97.60	83.3
103	102	103				102.67	50.0
		106	107	106	107	106.50	66.6
			111	111		111.00	33.3
116	114	114	116	114	116	115.00	100.0
128	126	126	126	127	128	126.83	100.0
			141	146	144	143.67	50.0
	156		153	156	156	155.25	66.6
			166	169	166	167.00	50.0
			186	185	184	185.00	50.0

Table 4.27 Migration distances of the gliadin subunits of SST 66 line 112.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Average	Repeat.
5		7				6.00	33.3
15	15	15	15	14	14	14.67	100.0
20		21	21		21	20.75	66.6
27	27	27	27	27	27	27.00	100.0
31	30	31	31	30	30	30.50	100.0
35	35	35	35	34	35	34.83	100.0
49	49	50	49	49	48	49.00	100.0
60	59	61	62	62	61	60.83	100.0
65	64	67	65	64	64	64.83	100.0
		73	71	70	70	71.00	66.6
77	78		78	79	78	78.00	83.3
86	87	84	85	85	83	85.00	100.0
89	90	87	89	89	89	88.83	100.0
92		92	93	93	94	92.80	83.3
95	96	96	99	98	98	97.00	100.0
101	102	102				101.67	50.0
106	105	106	107	106	106	106.00	100.0
113	113	112	118	119	119	115.67	100.0
124	123	123	126	124	127	124.50	100.0
133	133	131	128	128	130	130.50	100.0
148			147	148	146	147.25	66.6
	152	152	153		154	152.75	66.6
	164	162		166	167	164.75	66.6
	170	171		176	170	171.75	66.6
		186	185	186	186	185.75	66.6
193	193	192	192	193	193	192.67	100.0
198	198	198	199	200	200	198.83	100.0

Table 4.28 Migration distances of the gliadin subunits of SST 66 line 122.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Average	Repeat.
5		2	4	2		3.25	66.6
15	15	13	14	14	14	14.17	100.0
			21	21	20	20.67	50.0
27	27	25	27	27	26	26.50	100.0
30	30		30	30	30	30.00	83.3
35	34	33	34	34	34	34.00	100.0
			38	38	38	38.00	50.0
			44	43	45	44.00	50.0
49	49	48	48	47	49	48.33	100.0
60	59	62	61	60	60	60.33	100.0
65	65		64	64	63	64.20	83.3
	71		69	68	72	70.00	66.6
78	77			78	78	77.75	66.6
			82	82	83	82.33	50.0
84	85	84	84	84	85	84.33	100.0
87		89	88	87	88	87.80	83.3
90	91	92	90	91	91	90.83	100.0
96	94	94	92	95	95	94.33	100.0
101	101	100	96	98	99	99.17	100.0
			104	103	102	103.00	50.0
111	109	107	112	113	111	110.50	100.0
121	119	118	117	117	121	118.83	100.0
			126	126	125	125.67	50.0
			128	129		128.50	33.3
131			134	133	139	134.25	66.6
			143	143	143	143.00	50.0
	153		149	147	147	149.00	66.6
171			172	171	176	172.50	66.6
183			184	184	184	183.75	66.6
	186		186	186	186	186.00	66.6
188		188	188		188	188.00	66.6
196	194	197	193	193	193	194.33	100.0

Table 4.29 Migration distances of the gliadin subunits of SST 66 line 130.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Average	Repeat.
5	5	5	5	4	4	4.67	100.0
			10	10	11	10.33	50.0
13	14	14	15	16	16	14.67	100.0
19		20	20	20	22	20.20	83.3
26	27	27	27	28	29	27.33	100.0
29	30	30	31	32	32	30.67	100.0
34	35	35	35	35	36	35.00	100.0
50	51	52	51	52	51	51.17	100.0
65	67	67	62	65	60	64.33	100.0
	72	73	73	73	74	73.00	83.3
			75	75	75	75.00	50.0
80	81	81	81		80	80.60	83.3
84	84	86	84	84	83	84.17	100.0
89	87	90	88	90	88	88.67	100.0
92	92	92	93	93	91	92.17	100.0
97	99	99	99	100	100	99.00	100.0
101	102	102	102	103	103	102.17	100.0
110	111	111	110	110	110	110.33	100.0
121	122	122	121	117	117	120.00	100.0
129	131	131	128	128	126	128.83	100.0
		149	149	146	147	147.75	66.6
168	169	167	168		169	168.20	83.3
	173		171	171	172	171.75	66.6
177	176	176	181	181	180	178.50	100.0
184	185	186	185		185	185.00	83.3
188	188	189	189	190	190	189.00	100.0
197	196	196	194	199	199	196.83	100.0

Table 4.30 Migration distances of the gliadin subunits of SST 66 line 131.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Average	Repeat.
3	3	3	4	4	4	3.50	100.0
			8	8	9	8.33	50.0
14	13	13	15	15	15	14.17	100.0
	19	20	21	20	20	20.00	83.3
27	26	26	27	26	26	26.33	100.0
31	29	30	31	31	31	30.50	100.0
35	34	35	35	34	34	34.50	100.0
	47	43	40	40	40	42.00	83.3
53	52	51	52	50	49	51.17	100.0
60	57	60	54	54	62	69.40	83.3
67	66	66	67	66	66	132.67	50.0
75	78		77	72	75	75.40	83.3
81	81	83	83	80	80	81.33	100.0
88	88	87	88	87	86	87.33	100.0
			92	92	91	91.67	50.0
			94	96	95	95.00	50.0
98	97	97	99			97.75	66.6
103	102	100	102	103	103	102.17	100.0
111	110	109	109	108	107	109.00	100.0
	116	116	116	116	115	115.80	83.3
119	120	119	119		119	119.20	83.3
122	123		125		125	123.75	66.6
130	128		129	128	129	128.80	83.3
144	137	143	142	140	138	140.67	100.0
			150	151	151	150.67	50.0
156			157	157	156	156.50	66.6
181	179		180	180	179	179.80	83.3
184	183		187	188	186	185.60	83.3
195	195	195	200	198	200	197.17	100.0

Table 4.31 Migration distances of the gliadin subunits of SST 66 line 132.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Average	Repeat.
			9	8	8	8.33	50.0
13	12	13	14	15	14	13.50	100.0
26	25	26	25	25	25	25.33	100.0
29	28	30	29	29	29	29.00	100.0
34	33	34	33	33	32	33.17	100.0
	48	47		47	48	47.50	66.6
51	51	52	55	49	55	62.60	83.3
62		63	60	61	61	61.40	83.3
67	68	68	65	67	67	67.00	100.0
73	73	74	74	74		73.60	83.3
82	83	80	78	82	82	81.17	100.0
86	87		84	85	84	85.20	83.3
89	90	90	91	91	91	90.33	100.0
95		95	93	93	93	93.80	83.3
99	100	100	99	100	100	99.67	100.0
108	108	108	110	109	109	108.67	100.0
112			115	113	113	113.25	66.6
118	118	118	118	117	117	117.67	100.0
126	126	126	124	124	124	125.00	100.0
	135		135	133	135	134.50	66.6
			160	161	159	160.00	50.0
			165	168	165	166.00	50.0
			177	178	175	176.67	50.0
			184	183	181	182.67	50.0
			189	188	188	188.33	50.0
192	191	192	191	191	190	191.17	100.0
			197	197	197	197.00	50.0
			200	200	200	200.00	50.0

Table 4.32 Migration distances of the gliadin subunits of SST 66 line 134.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Average	Repeat.
4	5	4	4	4	4	4.17	100.0
10			9	7	9	8.75	66.6
14	14	14	15	15	13	14.17	100.0
21	20	20	20	20	20	20.17	100.0
27	28	28	25	26	27	26.83	100.0
30	31	31	30	30	30	30.33	100.0
35	36	36	33	34	34	34.67	100.0
			38	38	38	38.00	50.0
41	43		45	45	45	43.80	83.3
			48	48	50	24.33	100.0
52	53	53	53	55	55	64.20	83.3
62		63	62	58	61	61.20	83.3
69		67	66	66	69	67.40	83.3
74	76		75	72	72	73.80	83.3
81	80	80	81	82	82	81.00	100.0
83		84	85	85	85	84.40	83.3
87	87	88				87.33	50.0
90	92	92	91	89	89	90.50	100.0
100	98	98	101	99	99	99.17	100.0
104	102	102		103	102	102.60	83.3
			107	107	107	107.00	50.0
110	110	110		110	112	110.40	83.3
116	116	118	115	113		115.60	83.3
119	120		120	119	120	119.60	83.3
124	123		125	122	122	123.20	83.3
129	128		128	126	128	127.80	83.3
		131	133	132	133	132.25	66.6
			144	143	144	143.67	50.0
			149	147	147	147.67	50.0
			161	159	163	161.00	50.0
			171	169	171	170.33	50.0
			175	177	177	176.33	50.0
			181	181	181	181.00	50.0
			186	185	185	185.33	50.0
			188	190	189	189.00	50.0
193	193	193		194	193	193.20	83.3
			198		199	198.50	33.3
			200	200		200.00	33.3

Table 4.33 Migration distances of the gliadin subunits of SST 66 parent line.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Average	Repeat.
4	6		4	5	4	4.60	83.3
		15	15	15		15.00	50.0
	25	24	26	26	25	25.20	83.3
28	33	30	32	30		30.60	83.3
40	37	39		42	43	40.20	83.3
44	44	44				44.00	50.0
48	48	48				48.00	50.0
		51	51	51	51	51.00	66.6
58	59		62	60	62	60.20	83.3
70	70	70		70		70.00	66.6
75		75	75		75	75.00	66.6
83	85	82	85	81	82	83.00	100.0
94	93	90	89		90	91.20	83.3
99	98	96	96	98		97.40	83.3
	105	105	105	105		105.00	66.6
110	110	111	109	111	110	110.17	100.0
116	116	117	119	119	118	117.50	100.0
120	120			122		120.67	50.0
127	129	129	128			128.25	66.6
130	133	134		130	131	131.60	83.3
152	151	149	150	154	152	151.33	100.0
160		164	160	162	163	161.80	83.3
	172	174	173	170	172	172.20	83.3
181		185	182	182		182.50	66.6
194	195	194	194	196		194.60	83.3

Table 4.34 Migration distances of the gliadin subunits of Palmiet line 202.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Average	Repeat.
	4	4	12	9	9	7.600	83.333
17	16	16	18	19	18	17.333	100.000
27	28	28	28	28	27	27.667	100.000
30	31	31	31	31	31	30.833	100.000
36	36	36	35	36	35	35.667	100.000
52	53	52	49	50	49	50.833	100.000
63			64	64	63	63.500	66.667
68	68		68	68		68.000	66.667
	74	74	74	73	73	73.600	83.333
81		78	79	78	78	98.500	66.667
85	85	85		84	82	84.200	83.333
88	87	87	89		87	87.600	83.333
91	92	91	93	92	93	92.000	100.000
102	100	101	102	101	99	100.833	100.000
111	111	110		112	112	111.200	83.333
119	118	118	115		116	117.200	83.333
125	125	124	123	121	121	123.167	100.000
129	128	128	127	127		127.800	83.333
			133	134	131	132.667	50.000
149		152	149	153	149	150.400	83.333
163	161	163	160	160		161.400	83.333
	177		176	176	176	176.250	66.667
			181	182	182	181.667	50.000
			189	186	188	187.667	50.000
193	193	192	194	193	192	192.833	100.000
			200	200	200	200.000	50.000

Table 4.35 Migration distances of the gliadin subunits of Palmiet line 203.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Average	Repeat.
5	3	6				4.67	50.0
		11	12	14	15	13.00	66.6
16	15	17	18	23	23	18.67	100.0
27	26	28	27	32	33	28.83	100.0
35	34	35	24	28	40	32.67	100.0
51	48	50	57	50	52	51.33	100.0
65	65	65	60	63	66	64.00	100.0
	77	73	75	74	77	75.20	83.3
85	85	84		83	83	84.00	83.3
90	89	91		89	89	112.00	66.6
		96	96	94	94	95.00	66.6
99	100			101	102	100.50	66.6
108	109	105	104	107	105	106.33	100.0
		114	114	114	110	113.00	66.6
118	118	119	116		116	117.40	83.3
121	125		122	125		123.25	66.6
		129	128		128	128.33	50.0
	141		139	141		140.33	50.0
			150	149	150	149.67	50.0
156	152		153			153.67	50.0
	161		162	158	160	160.25	66.6
		168	165	167	167	166.75	66.6
			178	178	178	178.00	50.0
182	182	182	180	185		182.20	83.3
		188	187		188	187.67	50.0
191	191	196	198	195	194	194.17	100.0
	200	200	200	200	200	200.00	83.3

Table 4.36 Migration distances of the gliadin subunits of Palmiet line 207.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Average	Repeat.
11	11	11		14	12	11.80	83.3
16	17	16				16.33	50.0
23	21	21	23	21	20	21.50	100.0
28	27	27		27	25	26.80	83.3
31	30	30	33	31	31	31.00	100.0
36	35	34	36	34	34	34.83	100.0
51	50	49	53	53	51	51.17	100.0
65	65	64	67	67	66	65.67	100.0
73	73	73				73.00	50.0
77	77	77	78	77	77	77.17	100.0
84	84	83	83	83	81	83.00	100.0
87	87	85	88	87	87	86.83	100.0
91	91	90	93	93	92	91.67	100.0
			96	97	95	96.00	50.0
104	102		102	102	101	102.20	83.3
108	106	106	108	109	109	107.67	100.0
			113	111	112	112.00	50.0
115	115	116	118	116		145.00	66.6
124		124	125	127		100.00	83.3
131	130			131		130.67	50.0
135	137	138	139	138		137.40	83.3
167	168			165	167	166.75	66.6
181	184	185	179		181	182.00	83.3
191	190	192	193	190		191.20	83.3
200	200	200	200	199	198	199.50	100.0
191	191	196	198	195	194	194.17	100.0
	200	200	200	200	200	200.00	83.3

Table 4.37 Migration distances of the gliadin subunits of Palmiet line 208.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Average	Repeat.
3	2	2				2.33	50.0
4	5	4	7	7	7	5.67	100.0
12	11	9		9	9	10.00	83.3
16	15	15	18	17	16	16.17	100.0
21	20	21	23	23		21.60	83.3
27	26	30	29	27	26	27.50	100.0
33	34	33	36	35	34	34.17	100.0
48	47	47	50	48	48	48.00	100.0
64	65	61	65	65	64	64.00	100.0
72	70	70	74	71	70	71.17	100.0
76	76	76		76		76.00	66.6
78	80	80	80	80	79	79.50	100.0
82	83	84	83	83	85	83.33	100.0
89	88	88		89		88.50	66.6
93	93	93	94	94	95	93.67	100.0
103	103	104	102	103	102	102.83	100.0
107	107	106	106	107	107	106.67	100.0
110		110	110	112	111	110.60	83.3
115	116	115	116	116		115.60	83.3
124		128	123			125.00	50.0
135	135	135	132		135	168.00	66.6
159		159		164	160	160.50	66.6
168			167	167	170	168.00	66.6
			177	178	178	177.67	50.0
182	182	183	181	182	182	182.00	100.0
186	187	189			189	187.75	66.6
195	196	195	195	194		195.00	83.3
200	200	200				200.00	50.0

Table 4.38 Migration distances of the gliadin subunits of Palmiet line 211.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Average	Repeat.
10	4	11		7	9	8.20	83.3
15	15	16	16	15	16	15.50	100.0
25	26	27	27	25	26	26.00	100.0
29		29	30	29	30	29.40	83.3
33	33	35	35	34	34	34.00	100.0
44	47	49	49	49	50	48.00	100.0
64	65	66	66	64	65	65.00	100.0
72	71	71	71		72	71.40	83.3
76	75	77	77	75	75	75.83	100.0
82	82	81	81	81	83	81.67	100.0
85	88	89	89	86	89	87.67	100.0
98	97	97	97	99	93	96.83	100.0
102	103	104	104	103	101	102.83	100.0
107				108	105	106.67	50.0
117	116	117	117		112	144.75	66.6
	134	134	131			133.00	50.0
	138	139	137			138.00	50.0
174				176	174	174.67	50.0
	188	188	186	184	188	186.80	83.3
193	194	193	191	193	193	192.83	100.0

Table 4.39 Migration distances of the gliadin subunits of Palmiet line 212.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Average	Repeat.
			2	2	2	2.00	50.0
			9	9	10	9.33	50.0
16	16	17	16	17	17	16.50	100.0
21	21	22	21	21	22	21.33	100.0
28	28	28	26	27	27	27.33	100.0
30	30	31	30	31	31	30.50	100.0
35	35	36	34	35	35	35.00	100.0
40			46		44	43.33	50.0
50	51	51	49	50	50	50.17	100.0
61	61	62		60	58	60.40	83.3
67	65	66	66	65	67	66.00	100.0
72	72	74	71			72.25	66.6
78	78	78	77	78	77	77.67	100.0
82	83	82		83	84	82.80	83.3
85	84	85	85		86	85.00	83.3
91	91	92	93	95	95	92.83	100.0
100	99	99	102	104	101	100.83	100.0
			112	110	111	111.00	50.0
116	116	116	116	116	118	116.33	100.0
126	125	124		126		125.25	66.6
130	130	129				129.67	50.0
134	134	134	134	137	134	134.50	100.0
140	140	140			139	139.75	66.6
		146	145	143	143	144.25	66.6
160	163	163			162	162.00	66.6
170	177	177	175	174	173	174.33	100.0
185	182	182			185	183.50	66.6
190	189	187	190	190	189	189.17	100.0
197	195	193	199	200	198	197.00	100.0

Table 4.40 Migration distances of the gliadin subunits of Palmiet line 213.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Average	Repeat.
			3	2	3	2.67	50.0
	5		5	5	8	5.75	66.6
17	17	16	15	15	15	15.83	100.0
29	28	27	27	26	26	27.17	100.0
32	31	34	35	34	33	33.17	100.0
			49	48	47	48.00	50.0
52	52	50		54	53	52.20	83.3
66	65	63	65	64	64	64.50	100.0
	73	71	71	70	71	71.20	83.3
			75	75	75	75.00	50.0
80	84	84	83	82	80	82.17	100.0
88	90	90	89	88	90	89.17	100.0
92		92	93	92		92.25	66.6
96	97	96	95	94		95.60	83.3
99			98	99	99	98.75	66.6
104	102	102				102.67	50.0
	107		105	106	106	106.00	66.6
114	112	109	109	113	112	111.50	100.0
			118	120	120	119.33	50.0
124	122		124	123	124	123.40	83.3
129	127		128	126	127	127.40	83.3
133	135	134	134	136	134	134.33	100.0
144			145	145	145	144.75	66.6
	151	153	155	151	152	152.40	83.3
	160	158		160	158	159.00	66.6
	180	181		180	180	180.25	66.6
184	185		185	186	183	184.60	83.3
190		191	191	195	189	191.20	83.3
200	200	200	198			199.50	66.6

Table 4.41 Migration distances of the gliadin subunits of Palmiet parent line.

Repl. 1	Repl. 2	Repl. 3	Repl. 4	Repl. 5	Repl. 6	Average	Repeat.
5	5		5	5		5.00	66.6
23		23	23	23	23	23.00	83.3
34	32	34	34		36	34.00	83.3
39	39		38	39	40	39.00	83.3
55	54	54		55		54.50	66.6
	57	57		57		57.00	50.0
61		64	61			62.00	50.0
69	67	67	70	71	69	68.83	100.0
75	73	77	76	78	75	75.67	100.0
84		84	85	86	85	84.80	83.3
	97	97	97	97		97.00	66.6
105	103		106	107	106	105.40	83.3
113	114	113	114		113	113.40	83.3
118	118	119	117	117	118	117.83	100.0
136	133		135		135	134.75	66.6
144	144	145	144			144.25	66.6
162	165	164	165	166	165	164.50	100.0
167		167	168	168	168	167.60	83.3
175	176		177	177		176.25	66.6
180	182	181	181	181	180	180.83	100.0
186	187	187	188	187	187	187.00	100.0
200	200		200		200	200.00	66.6

Table 4.42 Summary of the lines tested and their different gliadin-subunit combinations, using the nomenclature system of Konarev (1979).

Cultivar	α	β	γ	ω
SST66 101	1;2*;4;6;7	4	3;4;5	1*;2*;3*;4;6;7*;8;9
SST66 102	1*;2*;4;6;	1;2;5	1;3;4;5	1*;2*;3*;4*;5;6;7;8*;9*;10
SST66 103	1;2;4;	5	1;4;	1*;2*;3;4;6;7;8*;9
SST66 105	1	1;2	1;3;5	1;2*;3;4*;6;7;8;9;10
SST66 112	1*;2;4;6	1;2;5	1;3;5	1*;2*;3;4;5;6;7*;8;9
SST66 122	1;2*;5	2;5	3;5	1*;2*;3*;4*;6;7*;8;9;10
SST66 130	1;2*;3;4;5	3	2*;4	1*;2*;3;4;5;7*;8*;9;10
SST66 131	1;2;3;7	3	2*;3*;5	1*;2*;3;4;5*;6;7*;8*;9;10
SST66 132	1	5	1;4*;5	1*;2*;3;4*;5;6;7;8*;9
SST66 134	1*	5	1;2;3*;4	1*;2*;3;4*;5;6;7*;8*;9;10*
SST66 parent	1;2;4;6	1;5	1;3;4;5	1*;2;4;5;6;8*;9;10
Palmiet 202	1;2;4;6; 7	4	5;4;3;1	1;2*;3;4;6;7*;8;9
Palmiet 203	1*;2;4;5	0	2;3;4;5	1*;2*;3;4;5;7,8;9*
Palmiet 207	1*;2;5	4	2;3;5	1*;2*;3*;4;5;7*;8*;9*
Palmiet 208	1*;2*;5;6	5	3;4;5	1*;2*;3*;4;6;7;8*;9*;10
Palmiet 211	1;2	5	4	1*;2*;3*;4*;6;7;8*;9;10
Palmiet 212	1;2*;4;6	3;4;5	1;4;5	1*;2*;3*;4;5;7;8*;9
Palmiet 213	1*;2*;7;	1;3;5;	1;2;4;5	1*;2*;3;4*;5;7*;8*;9;10
Palmiet parent	2*;3*;5;6	4;5	3;4;5	1;2;3;4;5;7*;8;10

The * is an indication of the presence of more than one band in the same interval.

The α-gliadin combination of bands 1 and 2 were found in almost all the lines. SST 66 lines 105, 132 and 134 had only α-gliadin band 1. Lines 101, 102, 112 and the parent line had the combination of bands 4 and 6. Line 103 had band 4 but not 6. Line 130 had the combination of 4 and 5, and also band 3. Bands 3 and 7 were found in line 131.

In the Palmiet cultivar the α-gliadin combinations looked very similar, with bands 1 and 2 found in all the lines except the parent line. The susceptible parent line had only band 2. Line 211 had only the 1 and 2 band combination. Combination 4 and 6 were found in lines 202 and 212. Lines 208 and the parent had a combination of bands 5 and 6, whereas in line 203 a combination of bands 4 and 5 were found. Line 213 was the only line with band 7.

The β-gliadin fraction had less combinations than the α-gliadin fraction. In SST66 lines 102 and 112 a combination of bands 1, 2 and 5 were found, while in lines 105 and 122, the combinations were 1 and 2, and 2 and 5 , respectively. In the SST66 parent line a combination of 1 and 5 were found. Lines 103, 130, 132 and 134 had only band 5. In SST66 lines 101 and 131 bands 4 and 3 were found, respectively.

In the Palmiet cultivar, five of the lines (208, 211, 212, 213, and the parent) had the β-gliadin band 5. The first two lines mentioned, lines 208 and 211 had only band 5, while the parent line had a combination of bands 5 and 4. Palmiet lines 212 and 213 had combinations of bands 3,4, and 5, and 1,3 and 5, respectively. Palmiet lines 204 and 207 only had band 4. Line 203 had none of the β-gliadin banding combinations.

The γ-gliadin banding patterns for the SST66 lines were very similar. SST66 lines 101, 102, 105, 112, 122, 131, and the parent line all had a combination of bands 3 and 5. Lines 102, 105, 112, and the SST66 parent also contained band 1. Lines 101, 102 and the parent line had band 4 between the 3-5 combination. SST66 line 103 had bands 1 and 4, while line 130 had bands 2 and 4. Lines 132 and 134 had combinations of 1,4,5 and 1,2,3,4 respectively.

Six of the Palmiet lines had the γ-gliadin pattern of bands 4 and 5. Only Palmiet line 211 had only band 4 from this fraction. Lines 202, 203, 208 and the Palmiet parent line had band 3 as well. Palmiet lines 202, 212 and 213 also contained band 1. Lines 203, 207 and 213 contained band 2.

The SST66 lines showed a very similar ω-banding pattern by containing most of the bands from this fraction. All the SST66 lines contained the bands from 1 to 4 and from 7 to 9. Except for the SST66 parent line in which band 7 were absent. In lines 101, 103, 105 and 122 band 5 was absent, while in line 130 band 6 was absent. In SST66 lines 101, 103, 112 and 132 band 10 was absent.

A similar pattern was observed for the Palmiet lines. Most of these lines also contained bands 1 to 4 and 7 to 9. Again, except for the Palmiet parent line, in which band 9 was absent. Lines 202, 208 and 211 did not have band 5 and in lines 203, 207, 212, 213 and the parent line band 6 was absent.

4.4. Discussion

4.4.1. HMW-GS

It was expected that all the progeny would contain the same banding pattern, seeing that they are NIL's (backcrossed 8-10 times). It was observed that most of the lines did have the same pattern, with the exception of four lines. The four lines (SST66 lines 130, 131 and Palmiet lines 208 and 213) with the exceptions observed are an interesting phenomenon, with the additional bands in their combinations making them hybrids. This was probably due to a small amount of segregation still occurring, even at this progressive stage of inbreeding.

4.4.2. LMW-GS

Most of the banding patterns had no deviation from the nomenclature currently used. Though a few exceptions were observed.

Chromosome 1A, group 1

The banding combinations observed in group one gives an indication of the subunits controlled by chromosome 1A. MacRitchie (1992) found this chromosome to have a very weak influence, with a very small number of bands controlled by this chromosome. The absence of bands in the e combination indicates that none of the bands are controlled by this chromosome. None of the cultivars or lines we examined had combination e.

Chromosome 1B, group 2

This group contains the highest number of bands with the most possible combinations. In agreement with the literature the greatest polymorphisms are shown by the 1B chromosome (MacRitchie, 1992; Gupta and Shepherd, 1990). Combinations d, h and i are very distinctive because of their double fragments in the B subunit. Most of the lines we tested had none of the double fragments. SST66 lines 103 and 130, however had the second set of double fragments, while Palmiet line 203 had the first double fragment set. Some of the lines also only needed one or two fragments of the C subunit to complete combinations g, h and I, respectively. Palmiet line 208 only has a possible combination e out of group two except for the fact that this line does not contain the slowest moving band of the C subunit. It is difficult to say if this is a new banding combination, or if the band was just too faint to be read. The last reason is the most probable, since the other bands are present.

Chromosome 1 D, group 3

In this test, deviations were also found in this group, indicating polymorphisms. The possibility of a new banding combination was observed. In six of the SST 66 lines, it was impossible to distinguish between the five combinations constituting this group respectively. In the Palmiet cultivar only lines 202, 212 and the parent line, were difficult to distinguish from one another.

In this group, SST66 lines 122 and 130, and Palmiet line 212 did not have the slowest moving band of the C subunit. SST66 line 131 had a last fragment moving slower than the accepted. This might be an indication of new banding pattern although confirmation is needed, although in the literature a combination named f is mentioned (Burridge and Cornish, 1993)

4.4.3. Gliadins

The results obtained are in agreement with the nomenclature. It is in agreement with the literature, some of the gliadin fractions are monomorphic. They appear in all the representatives of the species (Konarev *et al.*,1979).

The nomenclature however was not sufficient in some cases where more than one fragment appeared to be in the same interval. These cases are indicated in the summary with an asterisk after the band number. The most obvious double bands are present in the ω-fractions bands 1 and 2, this is true for both the SST66 and the Palmiet progenies. The Palmiet parent has no double bands present in either group, while the SST66 parent only has double bands in band 1. The same results were obtained for the α-fraction, with the progeny either having double fragments in band 1 and/or band 2. Some double bands were observed in the γ-fraction, but no double bands were visible in the β-fractions.

Large differences in the banding patterns for both the LMW-GS and gliadins of the NIL's were observed, despite the fact that these lines are almost 100% identical. In contrast to these findings, the banding patterns of the HMW-GS were almost identical for the progeny in the two genetic backgrounds. This proves, that while HMW-GS are insufficient for cultivar identification because of the similarities found in the banding patterns, both the LMW-GS and gliadins gave sufficient differences to enable distinction even between NIL's.

Further analyses are needed to determine the influences of the LMW-GS and the gliadin fragments on baking quality, since it differs from the results obtained HMW-GS for these identical genetic backgrounds.

Chapter 5

The use of Amplified Fragment Length Polymorphisms (AFLP) for marker detection of resistance to *Pseudocercosporella herpotricoides*.

5.1. Introduction

Eyespot is a chronic, widespread and economically important disease of cereal crops in temperate climates throughout the world. Severe infection with this fungus may result in crop losses of up to 50% (Scott and Hollins, 1974). The fungus is limited to the basal area of the stem, were the eyeshaped lesions are formed, weakening the stem and resulting in lodging (Murray and Bruehl, 1986).

Control of the fungus is very important to reduce yield losses. Fungicides have been commonly used to control the fungus. However, the use of fungicides are not cost effective or environmentally friendly. Resistant strains of the pathogen to fungicides have also been identified leading to renewed efforts of breeding for resistance (King and Griffin, 1985).

Four known sources of eyespot resistance have been identified, with the most effective resistance gene currently in use, being, *Pch1* (Cadle *et al.,* 1997). This gene, on the long arm of chromosome 7D, is derived from *T. ventrivosum* Ces and has subsequently been transferred to the wheat line "VPM-1", "Roazon" and "Rendezvous". An isozyme and RFLP marker for this resistant gene has been developed (Worland *et al.*, 1988).

The *Pch1* gene, however does not confer complete resistance. The fact that it is a single gene also increases the change of selecting for new virulent pathotypes

when using previously resistant cultivars. Gene pyramiding has thus been suggested. This involves the accumulation of a number of resistance genes into a single cultivar (Nelson, 1978)

The recently developed AFLP technique (Amplified fragment length polymorphisms) is proving to be very successful in DNA fingerprinting and molecular marker technology. This technique generates more data points per assay than any other available technique and therefore provides a greater capacity for large-scale mapping.

The aim of this study was to detect possible markers for eyespot resistance, in near isogenic resistant lines of Palmiet, with the use of the AFLP technique.

5.2. Materials and Methods

5.2.1. Plant material

The seed of seven near isogenic (backcrossed 8-9 times) resistant Palmiet lines were supplied by the ARC-SGI breeding programme in Bethlehem. These seeds were obtained from a cross between resistant Roazon, containing the *Pch* 1 gene, and the susceptible Palmiet parent. The seeds were propagated in the glasshouse.

5.2.2. DNA-extraction

DNA was extracted from the leaves of individual plants from the resistant lines as well as from the susceptible parent. A modified monocot extraction procedure (Edwards *et al.*, 1991) was used. Fresh leaves were collected on ice and ground to a fine powder in liquid Nitrogen. The grounded powder was then transferred to clean polypropylene tubes and 10 ml of extraction buffer (5M NaCl, 0.5M Tris-HCl, 0.25M EDTA and 20% SDS at pH 8) was added. The homogenate was vortexed and incubated at 65°C for 30 min. Clean-up buffer was added (1M Tris-

HCl, 0.25M EDTA and 5g of CTAB) and the extract incubated for a further hour at 65°C. Chloroform-isoamyl (24:1 v/v) alcohol was added, mixed gently and centrifuged for 15 min at 1000 rpm. The aqueous layer was transferred to a clean tube and the DNA precipitated with 100% cold ethanol. The DNA was spooled and washed twice with 70% ethanol. The DNA was sterile distilled water and concentrations determined using a spectrophotometer.

5.2.3. AFLP

AFLP analysis was performed using bulk segregant analysis (BSA). The DNA from the seven resistant Palmiet NIL's was bulked. The AFLPs reactions were done according to the manufacturers instructions (Gibco BRL).

5.2.3.1. Restriction Endonuclease digestion and ligation of adaptors

Genomic DNA (250ng) was digested with Mse1 and EcoR1. The digested fragments were then ligated with EcoR1 and Mse1 adapters (Table 5.1).

5.2.3.2. Polymerase chain reaction

A 51µl pre-selective PCR reaction was performed with 5µl diluted ligation product, pre-amp primer mix 10× PCR buffer and 1U of Ampli Taq DNA polymerase (GibcoBRL) was set up. A touchdown Hybaid thermal cycler was used to perform the reaction for 20 cycles with the following profile: a 30 s denaturing step at 94°C, a 60 s annealing step at 56°C and a 60 s extension step at 72°C. Pre-selective PCR products were diluted 50 fold in 1/50 TE.

Selective PCR-reactions were performed in a 20µl PCR reaction containing 5µl of the diluted pre-selective reaction, 4.5µl of the Mse+3 primer (Table 5.1), 1µl

Eco+3 (fluorescently labelled) , 2μl of 10×PCR buffer and 5U of Ampli Taq DNA polymerase. Reactions were performed for 30 cycles with the following cycle profile: a 30 s denaturing step at 94°C, a 30 s annealing step at 65°C and a 60 s extension step at 72°C. The annealing temperature is reduced by 0.7°C for 12 consecutive cycles and then continued at 56°C. A total of 16 primer combinations were tested. EcoR1 primers (PE Biosystems) were labelled with NED and FAM respectively.

Table 5.1 A list of adapter and primer sequences used in AFLP reactions the

Mse-adapter	**Eco-adapter**
5'-GACGATGAGTCCTGAG-3'	5'-CTCGTAGACTGCGTACC-3'
Mse-primers (5'-GATGAGTCCTGAGTAA-3')	**Eco-primers** (5'-GATGCGTACCAATTC-3')
Mse + CAA	Eco + ACA (FAM)
Mse + CAT	Eco + AAC (NED)
Mse + CTG	
Mse + CTT	
Mse + CTA	
Mse + CAG	
Mse + CAC	
Mse + CTC	

After amplification 5μl of each of the selective reactions were added to a new tube containing 24μl of formamide and 1μl of Rox standard size marker, denatured at 94°C for 5 min and run on a Perkin Elmer ABI Prism 310 Automated capillary sequencer (PE Biosystems).

5.3. Results

In this study, all the primer combinations tested yielded polymorphic results between the susceptible parental line and the bulked resistant lines. For primer combination Mse-CAA + Eco-ACA an average of 11 fragments were detected. The fragment sizes ranged from 72 to 432 bp. Six of these fragments (79, 116, 222, 225, 228, 432 bp) were specific for the resistant progeny and five (72, 147,

181, 367 bp) were only found in the susceptable parent (Table 5.2). Similarly for primer pair Mse-CAA + Eco-AAC an average of seven polymorphic fragments were detected whereof six (84,103, 110, 113, 121, 128 bp) were specific for the resistant progeny and one (367 bp) for the suceptable parent line (Table 5.3).

Using primer combination Mse-CAT + Eco-ACA an average of 14 polymorphic fragments were detected between the susceptable parent and resistant progeny. Ten (84, 86, 98, 103, 125, 137, 145, 165, 181, 205 bp) were specific for the resistant progeny and four (74, 131, 335, 690 bp) were only found in the susceptable parent (Table 5.4). Similarly using primer combination Mse-CAT + Eco-AAC an average of 17 polymorphic fragments were found whereof 8 (61, 96, 103, 137, 164, 405, 542, 595 bp) were only found in the resistant progeny and 9 (53, 88, 111, 136, 191, 318, 335, 372 bp) were specific for the susceptable parent (Table 5.5).

Primer combination Mse-CTG + Eco- ACA gave an average of 11 polymorphic fragments whereof 10 (37, 40, 60, 81, 91, 93, 140, 158, 163, 259 bp) were specific for the resistant progeny and one (50 bp) was found only in the susceptable parent (Table 5.6). For primer pair Mse-CTG + Eco-AAC an average of four polymorphic fragments were found whereof two (48, 232 bp) were specific for the resistant progeny and two (39, 55, bp) were specific for the susceptable parent (Table 5.7).

Using primer pair Mse-CTT + Eco-ACA an average of six polymorphic fragments were detected. Only two (48, 74 bp) of these were specific for the resistant progeny and the other four (156, 162, 293, 443 bp) were specific for the susceptible parent (Table 5.8). For primers Mse-CTT + Eco-AAC an average of 15 polymorphic fragments were detected whereof four (48, 113, 154, 195 bp) were found only in the resistant progeny and 11 (68, 106, 125, 135, 168, 181, 208, 213, 232, 295, 414 bp) were specific for the susceptible parent (Table 5.9).

Primers Mse-CTA + Eco-AAC gave an average of 15 polymorphic fragments whereof five (50, 242, 243, 331, 335 bp) were specific for the resistant progeny and 10 (35, 52, 78, 106, 119, 124, 139, 168, 172, 180, 194 bp) were found only in the susceptible parent line (Table 5.10). Similarly using primers Mse-CTA + Eco-ACA an average of seven polymorphic fragments were detected whereof four (47, 92, 331, 335 bp) were only found in the resistant progeny and three (67, 116, 131 bp) were specific for the susceptible parent Table (Table 5.11).

Using primers Mse-CAG + Eco-AAC an average of 21 polymorphic fragments were detected whereof 12 (72, 75, 78, 88, 110, 115, 128, 132, 149, 199, 206, 237, 276 bp) were only present in the resistant progeny and nine (43, 45, 47, 50, 53, 62, 101, 122, 176, 213 bp) were only found in the susceptible parent (Table 5.12). For primers Mse-CAG + Eco-ACA an average of eight polymorphic fragments were detected whereof six (76, 87, 105, 126, 175, 190 bp) were specific for the resistant progeny and two (93, 132 bp) were only found in the susceptable parent (Table 5.13).

Primers Mse-CAC + Eco-AAC gave an average of 18 polymorphic fragments whereof 15 (42, 65, 72, 80, 83, 91, 106, 119, 123, 152, 198, 218, 254, 299, 678 bp) were present in the resistant progeny and 3 (35, 50, 204 bp) were specific for the susceptable parent (Table 5.15). Similarly for primers Mse-CAA + Eco-ACA an average of 24 polymorphic fragments were detected whereof 7 (74, 125, 254, 350, 438, 473 bp) were specific for the resistant progeny and 17 (41, 43, 47, 49, 55, 59, 61, 65, 73, 102, 105, 117, 130, 146, 152, 198, 204, 233 bp) were found only in the susceptable parent Table(5.15).

Using primers Mse-CTC + Eco-AAC an average of 14 polymorphic fragments were detected whereof nine (106, 112, 119, 125, 132, 169, 191, 217, 222 bp) were specific for the resistant progeny and five (50, 53, 54, 78, 86 bp) were specific for the susceptable parent (Table 5.16). For primers Mse-CTC + Eco-ACA an average of 21 polymorphic fragments were detected whereof 17 (62, 73,

78, 103, 113, 129, 146, 176, 181, 185, 216, 222, 229, 233, 242, 286, 550 bp) were only found in the resistant progeny and nine (42, 49, 66, 170 bp) were spesific for the susceptable parent (Table 5.17).

Table 5.2. Polymorphisms observed between the bulked resistant NIL's and the susceptible parent line. Using primer combination Mse-CAA + Eco-ACA. Values are indicative of the presence of a polymorphic fragment as well as the fragment size in base pairs.

Resistant Palmiet progeny	Susceptible Palmiet parent
	72
79	
116	
	147
	181
	187
222	
225	
228	
	367
432	

Table 5.3. Polymorphisms observed between the bulked resistant NIL's and the susceptible parent line. Using primer combination Mse-CAA + Eco-AAC. Values are indicative of the presence of a polymorphic fragment as well as the fragment size in base pairs.

Resistant Palmiet progeny	Susceptible Palmiet parent
84	
103	
110	
113	
121	
128	
	367

Table 5.4. Polymorphisms observed between the bulked resistant NIL's and the susceptaible parent line. Using primer combination Mse-CAT + Eco-ACA. Values are indicative of the presence of a polymorphic fragment as well as the fragment size in base pairs.

Resistant Palmiet progeny	Susceptible Palmiet parent
	74
84	
86	
98	
103	
125	
	131
137	
145	
164	
181	
205	
	335
	690

Table 5.5. Polymorphisms observed between the bulked resistant NIL's and the susceptaible parent line. Using primer combination Mse-CAT + Eco-AAC. Values are indicative of the presence of a polymorphic fragment as well as the fragment size in base pairs.

Resistant Palmiet progeny	Susceptible Palmiet parent
	53
61	
	88
96	
103	
	111
	136
164	
	191
	210
	318
	335
	372
405	
542	
595	

Table 5.6. Polymorphisms observed between the bulked resistant NIL's and the susceptible parent line. Using primer combination Mse-CTG + Eco-ACA. Values are indicative of the presence of a polymorphic fragment as well as the fragment size in base pairs

Resistant Palmiet progeny	Susceptible Palmiet parent
37	
40	
	50
60	
81	
91	
93	
140	
158	
163	
259	

Table 5.7. Polymorphisms observed between the bulked resistant NIL's and the susceptible parent line. Using primer combination Mse-CTG + Eco-AAC. Values are indicative of the presence of a polymorphic fragment as well as the fragment size in base pairs.

Resistant Palmiet progeny	Susceptible Palmiet parent
	39
48	
	50
	55
	69
105	
232	

Table 5.8. Polymorphisms observed between the bulked resistant NIL's and the susceptible parent line. Using primer combination Mse-CTT+ Eco-ACA. Values are indicative of the presence of a polymorphic fragment as well as the fragment size in base pairs.

Resistant Palmiet progeny	Susceptible Palmiet parent
48	
74	
	156
	162
	293
	443

Table 5.9. Polymorphisms observed between the bulked resistant NIL's and the susceptible parent line. Using primer combination Mse-CTT + Eco-AAC. Values are indicative of the presence of a polymorphic fragment as well as the fragment size in base pairs.

Resistant Palmiet progeny	Susceptible Palmiet parent
48	
	78
	106
113	
	125
	135
154	
	168
	181
195	
	208
	213
	232
	295
	414

Table 5.10. Polymorphisms observed between the bulked resistant NIL's and the susceptible parent line. Using primer combination Mse-CTA + Eco-ACA. Values are indicative of the presence of a polymorphic fragment as well as the fragment size in base pairs.

Resistant Palmiet progeny	Susceptible Palmiet parent
	35
50	
	78
	106
	119
	124
	139
	168
	172
	180
	194
242	
243	
331	
335	

Table 5.11. Polymorphisms observed between the bulked resistant NIL's and the susceptible parent line. Using primer combination Mse-CTA + Eco-AAC. Values are indicative of the presence of a polymorphic fragment as well as the fragment size in base pairs.

Resistant Palmiet progeny	Susceptible Palmiet parent
47	
	60
92	
	116
	131
331	
335	

Table 5.12. Polymorphisms observed between the bulked resistant NIL's and the susceptible parent line. Using primer combination Mse-CAG + Eco-ACA. Values are indicative of the presence of a polymorphic fragment as well as the fragment size in base pairs.

Resistant Palmiet progeny	Susceptible Palmiet parent
	43
	45
	47
	50
	53
	62
72	
75	
78	
88	
	101
110	
115	
122	
	128
	132
	174
199	
206	
	213
237	
276	

Table 5.13. Polymorphisms observed between the bulked resistant NIL's and the susceptible parent line. Using primer combination Mse-CAG + Eco-AAC. Values are indicative of the presence of a polymorphic fragment as well as the fragment size in base pairs.

Resistant Palmiet progeny	Susceptible Palmiet parent
76	
87	
	93
105	
126	
	132
175	
190	

Table 5.14. Polymorphisms observed between the bulked resistant NIL's and the susceptible parent line. Using primer combination Mse-CAC + Eco-ACA. Values are indicative of the presence of a polymorphic fragment as well as the fragment size in base pairs.

Resistant Palmiet progeny	Susceptible Palmiet parent
	35
42	
	50
65	
72	
80	
83	
91	
106	
119	
123	
152	
198	
	204
218	
254	
299	
678	

Table 5.15. Polymorphisms observed between the bulked resistant NIL's and the susceptible parent line. Using primer combination Mse-CAC + Eco-AAC. Values are indicative of the presence of a polymorphic fragment as well as the fragment size in base pairs.

Resistant Palmiet progeny	Susceptible Palmiet parent
	41
	43
	47
	49
	55
	59
	65
	73
74	
	102
	105
	117
125	
	130
	146
	152
	198
	204
	233
254	
350	
438	
473	

Table 5.16. Polymorphisms observed between the bulked resistant NIL's and the susceptible parent line. Using primer combination Mse-CTC + Eco-ACA. Values are indicative of the presence of a polymorphic fragment as well as the fragment size in base pairs.

Resistant Palmiet progeny	Susceptible Palmiet parent
	50
	53
	54
	78
	86
106	
112	
119	
125	
132	
169	
191	
217	
222	

Table 5.17. Polymorphisms observed between the bulked resistant NIL's and the susceptible parent line. Using primer combination Mse-CTC + Eco-AAC. Values are indicative of the presence of a polymorphic fragment as well as the fragment size in base pairs.

Resistant Palmiet progeny	Susceptible Palmiet parent
	42
	49
62	
	66
73	
78	
103	
113	
129	
146	
	170
176	
181	
185	
216	
222	
229	
233	
242	
286	
550	

5.4 Discussion

A total of 16 primer combinations were used to fingerprint both the bulked Palmiet resistant progeny (NIL'S) and the susceptible parent. Distinct differences were found between the resistant NIL and the susceptible parent in fragment polymorphism. These differences can be attributed to the introduced translocated segment from the donor parent.

It is interesting to note that the polymorphisms over all the primers tested between the resistant and susceptible lines is 37.11%. The fact that so many polymorphisms are detected between the susceptible parent line and the resistant NIL's may be the result of a large donor segment. The Pch1 gene is situated on the long arm of chromosome 7D (Cadle *et al.*, 1997). The co-introgressed region may lead to the identification of false markers, so it is important to test the linkage of the possible markers in segregating populations.

Of the possible markers identified, only fragments larger than 100 bp are considered suitable for further testing and development. In total, 83 fragments from the different primer combinations are considered suitable for further development into STS (sequenced-tagged sites) markers. This is done by sequencing the fragments and designing PCR primers based on these sequences. STS provide highly reproducible and informative single-locus codominant markers.

Chapter 6

Conclusions

The wheat industry is of major economical importance. The most important decision a farmer has to make each year is which cultivar to grow, to ensure a good harvest. Until recently, high yield was the most important selection criteria, but the quality attributes are becoming increasingly important. One can understand the farmers apprehension, when a new pathogen resistant cultivar is released. Especially if they do not have the prove, that the resistance was obtained without detrimental effect on the yield or quality of the wheat, in the absence of disease infections.

The first objective of this study was to determine whether the presence of the gene for eyespot resistance, had any effect on the baking quality of the Palmiet and SST66 lines, tested. Quality traits are mainly polygenic and are influenced by both the environment and the genotype. A change in the genetic constitution of plants can thus have a major effect on one or all the quality attributes.

The presence of the gene had no effect on the quality or yield aspects of the wheat. The presence of the gene, thus 1) protects the plant without 2) any detrimental effect on yield or quality. The implication of this is that farmers can plant resistant cultivars with confidence, without fear of compromising baking quality.

The HMW-GS, LMW-GS and gliadin subunit banding combinations for the 10 SST66 and the seven Palmiet resistant lines, as well as for their respective susceptible parents were determined. These techniques can be used to screen

early generations for use in breeding programmes, and also for cultivar identification and homozygosity tests.

Due to a limited amount of variability in the HMW-GS it will be a very effective method, which can be used to screen wheat cultivars for homozigosity as far as wheat quality is concerned. It is not an effective method to use to distinguish between different wheat cultivars. This makes it insufficient for use in cultivar identification and homozygosity determination. The LMW-GS showed more genetic variation between the resistant (NIL's) and susceptible cultivars than the HMW-GS. This makes the LMW-GS more suitable for cultivar identification and homozygosity determination than the HMW-GS. It is best, however to use both or to develop an identification system based on both.

The results showed that the LMW-GS and the gliadins showed still a large amount of variability after eight to ten generations of backcrossing. Therefore the chance that LMW-GS and gliadins will reach homozygosity after four to six generations of inbreeding is almost none. Therefore it cannot be used to screen for homozygosity. However it will be an effective method to use to distinguish between different cultivars for uniqueness.

Using the AFLP method, distinct differences were found between the resistant NIL's and the susceptible parents. This may be the result of a large donor fragment. Several putative markers for *Pch 1* were identified in the Palmiet background. Of the possible markers only 83 fragments will be considered for further testing and development to STS markers.

Chapter 7

Summary

1. The aim of this study was to determine whether the presence of the *Pch1* gene, has an effect on breadmaking quality and yield in two genetic backgrounds, to find a molecular marker linked to the gene, using the AFLP technique and to look at the protein profiles for the NIL's and their recurrent parents.

2. Seed from near-isogenic resistant Palmiet and SST66 lines were obtained from the ARC-SGI in Bethlehem. ANOVA's showed that the presence of the gene had no significant effect on yield.

3. Baking quality tests were done at the SGI-ARC in Bethlehem to study the effect of the gene on the quality traits. The results showed no significant differences occurred between the susceptible parents and the resistant progeny lines with regard to wheat quality.

4. The HMW-GS, LMW-GS and gliadin banding patterns for the different lines were also studied. The results obtained showed that the HMW-GS were inadequate to distinguish between NIL's, while the LMW-GS and the gliadins gave effective distinction between the lines. Further analyses are needed to determine the effects of the subunits on the baking quality.

5. Using the AFLP technique we were able to detect possible markers between the susceptible parent lines and the bulked resistant progeny. Further tests are needed to determine the degree of linkage between the possible markers and the *Pch*1 gene.

Opsomming

1. Die doel van die studie was om die effek van die *Pch1* geen op die bakkwaliteit en opbrengs in die twee genetiese agtergronde vas te stel, om 'n molekulêre merker te identifiseer deur van die AFLP tegniek gebruik te maak, en om die proteïen profiele van die NIL's en hul herhalende ouer te bestudeer.

2. Saad van naby-isogeniese weerstandbiedende Palmiet en SST66 is vanaf die KGI, Bethlehem verkry. Die saad is in 'n gerandomiseerde blok ontwerp geplant en die opbrengs is met behulp van ANOVA's bepaal. Dit is duidelik uit die resultate dat die geen nie opbrengs beinvloed nie.

3. Die bakkwaliteittoetse is in die laboratorium van die KGI, Bethlehem uitgevoer. Die resultate het getoon dat die teenwoordigheid van die weerstandsgeen geen betekenisvolle invloed op die bakkwaliteit het nie.

4. Toetse om die HMW glutenien, LMW glutenien en die gliadien samestelling van die NIL's in die twee genetiese agtergronde vas te stel is ook gedoen. Uit die studie was dit duidelik dat die HMW glutenien onvoldoende is om tussen naby-isogeniese lyne te onderskei. Die LMW gluteniene en die gliadiene het meer verskille tussen die lyne aangedui. Verdere studies is egter nodig om die effek van die verskillende fragment kombinasies op die bakkwaliteit te bepaal.

5. Die AFLP-resultate het 'n groot aantal polimorfismes tussen die vatbare ouer en die weerstandbiedende nageslag aangetoon. 'n Moontlike rede

hiervoor is die grootte van die translokasie fragment. Die moontlike merkers moet verdere toetse ondergaan om ten volle ontwikkel te kan word.

Chapter 8

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