Genotype effect of South African barley cultivars on malting quality under different nitrogen levels

By

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Thesis submitted in accordance with the requirements for the Magister Scientiae Agriculturae degree in the Faculty of Natural and Agricultural Sciences, Department of Plant Sciences (Plant Breeding), at the University of the Free State, Bloemfontein

University of Free State
Bloemfontein
June 2009

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DECLARATION

I declare that the thesis hereby submitted by me for the **Magister Scientiae Agriculturae** degree at the University of the Free State is my own independent work and has not previously been submitted by me at another university/faculty. I further concede copyright of the thesis in favour of the University of the Free State.

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ACKNOWLEDGEMENTS

I wish to convey my sincere gratitude and thanks to the following individuals for their contributions to the completion of my MSc study and preparation of this thesis:

- My study leaders, Dr Elfranco Malan, Prof Maryke Labuschagne and Dr Angie Van Biljon for their advice, invaluable inputs and especially for their time. I appreciate your confidence in me and encouragement throughout my studies.

- Mr Wiempie du Toit and Mr Manus van der Merwe and their teams at Vaalharts and Rietriver for the maintenance of my field trials.

- Mr Barend Wentzel and Mrs Marie Erasmus for their assistance with the HPLC and Leco analysis.

- Prof Maryke Labuschagne and Mrs Marie Smith for their assistance with the statistical analysis.

- All my colleagues at ARC-Small Grain Institute for their continuous support and help towards the completion of this thesis.

- Mrs Sadie Geldenhuys for her assistance with the administrative work associated with my studies.

I want to express my heartfelt thanks to my family and friends for their constant support and encouragement throughout my studies.

Also a special thanks to the NRF and ARC-Small Grain Institute for financial support.

LIST OF ABBREVIATIONS

ACN	=	acetonitrile
ANOVA	=	analysis of variance
AU	=	arbitrary unit
cc	=	cubic centimeter
cv	=	coefficient of variance
G x E	=	genotype by environment interaction
GA_3	=	gibberellic acid
kg/ha	=	kilogram per hectare
LTm	=	light transmission meter
nm	=	nanometer
N	=	nitrogen
NCSS	=	number cruncher statistical system
P	=	probability of significance
QTL	=	quantitative trait locus
RP-HPLC	=	reverse phase-high performance liquid chromatography
t/ha	=	ton per hectare
TFA	=	trifluroacetic acid
µl	=	microlitre
µm	=	micrometer
UV	=	ultraviolet

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CHAPTER 1
General introduction

1.1 Malting barley

Barley (*Hordeum vulgare* L.) is the most widely used cereal crop in the malting, brewing and feed industries in the world (Brennan *et al*., 1997). With increasing beer consumption in the world there is a high demand for malting barley (Sardana and Zhang, 2005a). In South Africa, barley is mainly produced in the Western Cape (70%) and Southern Cape (3%) under dry land conditions and in the Northern Cape (27%); Taung and Vaalharts areas under irrigation conditions. Due to the unfavourable climate conditions in the Western and Southern Cape, substantially more barley is produced in the Taung and Vaalharts areas. In recent years barley production in South Africa has not met the parameters for good malting quality, as a result malt barley had to be imported from Canada, the United States, Denmark and France (Anonymous, 2001). Thus considerable efforts are required in South Africa to increase malt barley production and to minimize dependence on other countries (Sardana and Zhang, 2005a).

Barley is commonly used for malting as it has a three-celled aleurone layer that ensures extensive and uniform breakdown of the starchy endosperm which is important in the production of good quality malt (Brennan *et al*., 1997). The malting quality of barley is very complex and is controlled by many genes and is strongly influenced by the environment (Fox *et al*., 2003). The most important quality parameters for the malting industry include plump kernels (>2.5 mm), protein content in the range of 9 - 12%, kernel nitrogen (N) concentration between 1.5 and 1.95%, high diastatic power and high malt extract (De Ruiter, 1999; Grausgruber *et al*., 2002).

One of the main concerns in the barley industry is the need to implement good N fertilizer management systems to obtain good malting quality and high yield. Plants obtain N from the soil and the fertilizer applied. The rate and timing of N fertilizer application is important for good crop development. Various studies have been conducted and have shown that fertilizer should be applied at sowing to encourage crop and tiller development and at the end of tillering to enhance the yield and be used as a

sink (accumulation of nutrients) during post-anthesis (Baethgen *et al*., 1995; Sardana and Zhang, 2005a).

In South Africa, a minimum of 120 kg/ha of N is required for optimum barley yields under irrigation (Kotzé, 2005). Insufficient N reduces grain yield and excessive N increases fertilizer costs, causes lodging and also has a negative effect on yield and may result in a high grain protein level, which is unacceptable as malting quality is affected. The plant takes up N from the five-leaf stage until heading and this results in an increase in yield. From heading to two weeks after flowering N has minimal effect on yield but can increase protein content of the grain (Ottman and Thompson, 2006). Grain yield can only be increased by adding optimum levels of N fertilizer, beyond the optimum N level, grain yield will decrease as a result of a decrease in kernel plumpness, enzyme activities, extractable malt and diastatic power (Thompson *et al*., 2004).

1.2 Aims of this study

1. To study the relationship between leaf and kernel N after N fertilizer application at a particular growth stage.
2. To determine the influence of N fertilizer applications on malting quality traits.
3. To determine if there are differences within the doubled haploid populations in their response to the different N fertilizer applications for malting quality.
4. To determine the influence of the N fertilizer applications on the production of storage proteins (hordeins) with reverse phase-high performance liquid chromatography (RP-HPLC) analysis.
5. To estimate the differences in the double haploid populations for their ability to produce these proteins by using RP-HPLC analysis.
6. To determine with RP-HPLC if proteins are highly correlated with malting quality traits.

1.3 Hypothesis

It is possible to manipulate N content in barley grains to ensure good malting quality by applying N at different stages of plant growth development.

CHAPTER 2

Literature review

2.1 Barley and malting quality

2.1.1 Structure of the barley plant

Barley (*Hordeum vulgare* L.) belongs to the monocotyledonous grass family Poaceae (Manninen, 2000). The barley plant has two stems namely: a main stem and lateral branches or tillers. At anthesis (flowering), plants possess a main stem and one to three primary tillers and at harvest an average of five stems per plant could be attained. There are two main types of barley based primarily on the number of kernel rows namely, two-row and six-row barley. Each type has three spikelets at each rachis node (one central and two lateral), and each spikelet consists of two glumes and one floret (Figure 2.1). In two-row barley the lateral spikelets are sterile and in six-row barley all three spikelets may be fertile, each fertile floret has three stamens and a pistil enclosed in the lemma and palea (Wiebe and Reid, 1961; Foster, 1987).

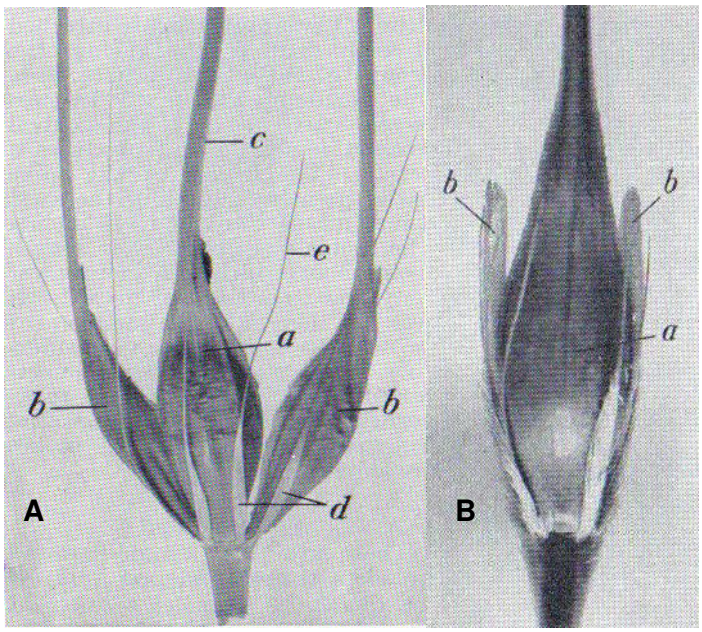

Figure 2.1. A. Spikelet of six-row barley: a, Central kernel; b, lateral kernels; c, awn; d, glumes; e, glume awn. B. Spikelet of two-row barley: a, Central kernel; b, lateral florets, sterile (Wiebe and Reid, 1961)

Stages of grain development include initiation of the spikelet, flowering, grain growth and maturation (Ellis and Marshall, 1998). Anthesis begins at the centre of the spike and proceeds to the top and bottom. The timing of anthesis depends on the genotype and

the environment. Barley can further be classified as spring or winter types, hulled or hulless and as malting or feed types (Wiebe and Reid, 1961; Foster, 1987).

Two-row barley further differs in nutritional properties from six-row barley in that two-row barley has low enzyme content, fewer proteins, more starch which contributes to more malt extract and a thinner husk which generally has lower levels of polyphenols (tannins) which gives the beer a less bitter taste. Six-row barley has a higher enzyme content, more proteins which gives the finished beer a haze, less starch and lower malt extract, a thick husk with a greater amount of tannins resulting in a bitter tasting beer. Two-row barley is therefore used for malt and six-row barley for animal feed (Goldhammer, 2000).

Barley is a self-pollinating crop so there is little scope for genetic variation due to out-crossing (Ellis and Marshall, 1998). New barley cultivars are developed by crosses made between adapted high yielding cultivars and breeding lines. This is followed by identification and selection of desirable characteristics such as yield, disease resistance and malting traits. After the crosses are made, selection of traits is difficult in the early generations as the populations are highly heterozygous (Manninen, 2000). This problem can be overcome by producing doubled haploids where homozygosity can be reached in one generation (Foster, 1987).

2.1.2 The malting process

The malting process includes the breakdown of starch, protein and nucleic acid molecules in barley grains into sugars, amino acids and nucleotides (Swanston *et al*., 1995; Jones, 2005). There are three stages during malting, namely steeping, germination and kilning. Firstly, the barley grains are steeped (soaked) in water to remove dirt and microbes and the moisture content within the grains are raised to promote germination. Secondly, germination of grains occurs under controlled temperature and moisture conditions. The enzymes produced during germination break down starch into the sugar maltose, which is then fermented by yeast to produce alcohol and carbon dioxide (Jones, 2005). Lastly, the grains are dried by heat to reduce moisture, preserve enzyme activity and develop colour and malting flavour (a process called kilning) (Hayes *et al*., 2003).

The breakdown of the cell walls and the protein matrix of the starchy endosperm during malting are known as modification (Wentz *et al*., 2004). Uniform and extensive modification of the kernels is very important when determining malt quality, in order to obtain homogenous malt (Reinikainen *et al*., 1996). Barley endosperm cell walls are composed of 1.3 and 1.4 β-D-glucans. The cell wall is broken down by endo-β-glucanases and this process is important for extract development during malting. Therefore the amount of β-glucans in the cell wall and their ability to synthesize endo-β-glucanases can be used to determine the rate of endosperm modification of barley samples (De Sá and Palmer, 2004). Malting quality of barley, however, requires a low percentage of 1.3 and 1.4 β-glucan and protein contents. High levels of β-glucans (>4.6%) limit the rate of modification of the endosperm. Studies showed that β-glucan content may be affected by both environmental and genetic conditions (Zhang *et al*., 2001).

Two factors that should be taken into consideration for barley malt modification are: Firstly, the physiological factor which is the entry of β-amylase to the aleurone layer for modification. Secondly, the structural factor which may cause resistance to modification (Munck and Møller, 2004). A mealy endosperm is preferred over a steely endosperm as it is less densely packed which allows water to penetrate more easily, which is needed for enzymatic activity during modification. The light transmission meter (LTm) gives a good indication of the endosperm structure of barley. It is based on the principle that more light will pass through and be scattered in a mealy endosperm since it is less dense compared to a steely endosperm, which is more dense (Chandra *et al*., 2001). The Calcoflour method, which makes use of the Carlsberg Calcoflour stain and image processing, can also be used to determine malt modification and homogeneity. This method is useful as it can eliminate the possibility of human error (Reinikainen *et al*., 1996). The flotation method can be used to select fast modifying grains on a large scale, using salt solutions showing the densities of the grains. The less dense or mealy grains with low nitrogen (N) content will float compared to the denser grains with high N content (Briggs *et al*., 2001).

2.1.3 Malting quality

Maltsters use three characteristics namely, kernel plumpness, protein levels and germination rate as indicators of malting quality when they purchase barley (Mather *et al*., 1997). Uniform grain quality and high malt yield are very important for malting. The relationship between yield and quality is affected by soil fertility, cultivar, N management, and soil water availability and by patterns of N uptake at pre- and post-anthesis. The application of N and irrigation practices may influence malting quality in the field (De Ruiter, 1999).

2.1.4 Grain size and weight

Grain size is an important trait for both malt and feed quality. With plump grains, a higher malt extract can be obtained to ensure good malt quality and more starch per grain can be obtained for good feed quality. Long and narrow kernels produce lower malt extract, impede water absorption during steeping and have a higher protein content and low starch content (<21% amylose) (Swanston *et al*., 1995) compared to short and plump kernels. However, really large kernels may affect the rate of water hydration and modification during malting (Fox *et al*., 2003).

Grain size is determined by the grains retained on 2.8 mm, 2.5 mm and 2.2 mm sieves respectively. Kernel plumpness is determined by the percentage of grain >2.5 mm and thinner grains or screenings is the percentage of <2.2 mm grains (Gebhardt *et al*., 1993; Coventry *et al*., 2003). Two-row barley has plumper grains than six-row barley and 85% kernel plumpness is required for two-row barley and 70% for six-row barley. There is unacceptable variation in grain size of six-row barley and hence it is used for animal feed and not for malt barley (Ellis and Marshall, 1998).

Grain size and weight is complex and controlled by many genes, of which their quantitative loci's (QTL's) are scattered throughout the barley genome. These traits are both influenced by abiotic and biotic stress, such as water availability and temperature, as well as agronomic or morphological effects. An understanding of genetics and environmental influence on grain size and weight is important (Coventry *et al*., 2003) and to maintain both traits across environments both are often main goals in breeding

programmes. Grain weight is a component of grain yield and also determines malting quality of barley. The amount of carbohydrates available during grain filling and the duration of the grain filling period are the main factors contributing to grain weight. It is determined per 1000 kernel weight (Ferrio *et al.*, 2006).

2.1.5 Germination

The barley grain is made up of the embryo, seed coat, aleurone layer and starchy endosperm. The starchy endosperm is further divided into the sub-aleurone layer, the prismatic and central regions (Figure 2.2) (Brennan *et al.*, 1996; Koning, 2006). Water and aerobic conditions are necessary for germination (Briggs, 2002) and in the presence of water the embryo secretes gibberellic acid (GA_3) into the cells of the aleurone layer. Gibberellic acid induces the synthesis of α-amylase in the aleurone cells. The amylase is transported from the aleurone cells into the endosperm where they break down starch to the sugar maltose which supports the growth of the embryo (Jenson, 1994; Yan *et al.*, 1999; Goldhammer, 2000; Koning, 2006).

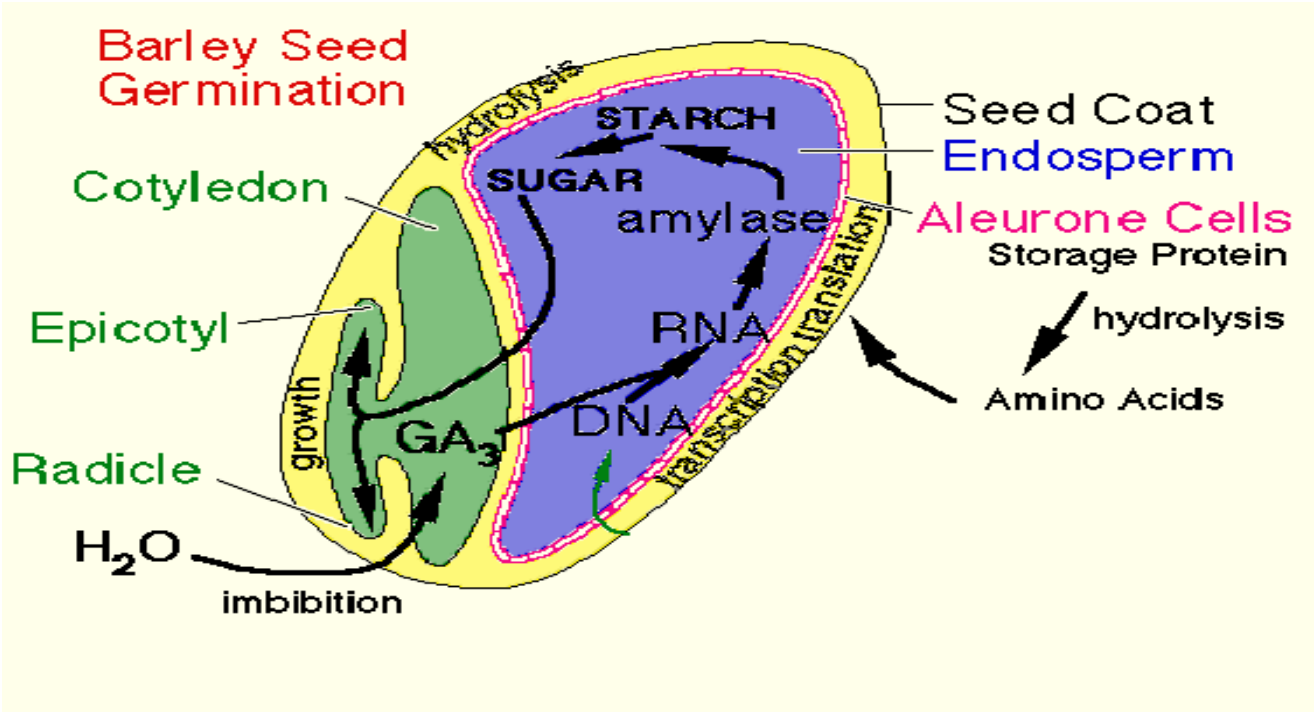

Figure 2.2. The process of germination of a barley seed (Koning, 2006)

Germination is complete when the radicle protrudes from the grain. Uniform germination of the kernels is important for the production of malt for beer brewing (Fox *et al*., 2003). Malting quality is therefore affected by uneven modification of the endosperm, as well as rates of modification and by non-germinated kernels. Slow germinating kernels are not desired, as they convert starch to sugars at a slow rate and as a result will reduce the yield of malt extract. Pre-germination of seed may also occur due to unfavourable environmental conditions and could result in non-homogenous malt. Although malt extract is a good trait to select for in early generations, probably because it has reduced variability, it would be valuable to assess malting quality without the need to malt (Reinikainen *et al*., 1996).

Germination rate is an important character for seed and malting quality, and is evaluated under optimal conditions for germination, i.e. at laboratory temperatures (20ºC) and at optimal moisture (<15%) (Chloupek *et al.,* 2003; Fomal and Filipowicz, 2005). The barley kernels germinate for 72 h and the germinated kernels are then called malt (Yan *et al*., 1999). Vigour of barley seeds is determined in 24 h of germination and viability in 72 h. High vigour is a good indication of fast and efficient germination capacity, which is desired by the malting industry (Munck and Møller, 2004). Germination of kernels under controlled conditions in the laboratory may differ from germination under field conditions and during malting procedures. This may occur when field conditions are unfavourable for germination and test results will therefore not correlate with field emergence (Chloupek *et al*., 2003).

2.1.6 Grain protein content

Grain protein content is also important for malting quality and affects water uptake, germination and modification during malting (Sardana and Zhang, 2005b). The South African maltsters require grains with protein content in the range of 9 - 12%. High grain protein is correlated with low carbohydrate content and low malt extract, thus prolonging the malting process and affects the final beer quality (Zhang *et al*., 2001). Low grain protein results in limited amino acids available for yeasts during brewing (Fox *et al*., 2003). It is difficult to obtain consistent grain protein content in the specified range because of low heritability and the influence of genotype x environmental interaction (G x E) (Emebiri *et al*., 2003). Grain protein content is affected by the rate and time of N

fertilizer application, available N in the soil (Chen *et al*., 2006), water availability and temperature (Riley *et al*., 1998).

Protein content is usually measured as total N using the Kjeldahl method. However, the Dumas method has proved to be valuable in determining total N content of single barley kernels (Anonymous, 1987). Breeders can use this method as a quality parameter to determine homogeneity within new barley cultivars. The Dumas method uses high combustion temperature followed by reduction of nitrogenous compounds to elemental N, which is measured by a thermal conductivity detector. Advantages of this method over the Kjeldahl method is that almost all N (nearly 100%) is recovered in a short time that is, 10 minutes per sample, only a small sample size is required and it eliminates the use of harmful and toxic chemicals. The small sample size makes quality selection in early generations possible for breeders (Angelino *et al*., 1997).

2.1.7 Grain nitrogen content

Grain N is a measure of proteins, and is an important guide for other quality parameters (Carreck and Christian, 1991) and many genes play a role in grain N content (Foster, 1987). There are different N requirements for the different types of beer with the N concentrations varying between 1.5 - 1.95%. This variation can be due to environmental conditions such as low radiation and high temperature especially when the grain filling period is lengthened and more N is taken up from the soil (De Ruiter, 1999) and if the crop experiences heavy rainfalls, leaching of nutrients can occur which could affect plant growth development (Baethgen *et al*., 1995). High N content has been linked with uptake of soil N during grain filling. However, continued uptake of N from the soil may not be detrimental due to soil moisture content (De Ruiter, 1999). Less N tends to decrease the number of grains per plant but has little effect on individual grain weight and in some cases may lead to higher grain weight due to compensation mechanisms (Ferrio *et al*., 2006).

2.2 Nitrogen fertilizer management

Fertility is an important factor that affects both quality and yield of a crop. It is important to know the plant's nutritional status during the growing season to manipulate these

important parameters. Plant tissue analysis is used to determine the nutritional status of plants and analysis will detect nutrient deficiencies and toxicities which can be confirmed by the physical appearance of the plants and allow correct fertilizer management. Both plant tissue analysis and soil analysis are important tools in determining the nutrient requirements of a crop and are therefore valuable tools for N fertilizer management (Flynn *et al*., 1999; Ottman and Thompson, 2006).

Tissue testing is not frequently used to determine the N status of a crop because of delays that occur between collection of samples and completion of chemical analysis and it is an expensive technique (Wright *et al*., 2004). However, a faster and easier approach is the use of chlorophyll meters for N management. Since most leaf N is contained in chlorophyll molecules, there is a strong relationship between leaf N and leaf chlorophyll N. The relative chlorophyll content can be used to predict N status of the crop and to predict yield and quality of the crop (Izsáki and Németh, 2007). The chlorophyll meter is based on the principle of the ability of chlorophyll to absorb red light and N is determined by the amount of red light absorbed. The more red light absorbed, the more chlorophyll is present and the greener the plant. The factor limiting the use of chlorophyll meters for N crop management is that the meter cannot indicate how much excessive N is available to the crop (Francis and Piekielek, 2007).

Nitrogen accumulated during the vegetative period contributes 20 - 70% of the final N seed yield. Leaves and stems mobilize more than 65% of their N to the seeds. Rubisco (ribulose-1,5 biphosphate carboxylase/oxygenase), which constitutes 50% of the total protein content in leaves, is thought to be a major source of N for mobilization (Lea and Azevedo, 2006). In monocarpic species, such as barley (Lammer *et al*., 2004), N mobilization during grain filling is related to senescence of vegetative parts. Senescence is associated with a decrease in protein and chlorophyll followed by leaf yellowing. Degradation of leaf cell constituents allows relocation to plant sinks (Schiltz *et al*., 2004).

Ammonia forms of N are better than urea or nitrate forms since it will not leach past the limited root system of the young plants. Aqua or anhydrous forms of ammonia, however, may injure plants due to ammonia toxicity (Ottman and Thompson, 2006). Although the application of nitrates to barley primary roots results in the formation of lateral roots, too much nitrate retards root growth to 0.2 - 0.5 mm in length (Lea and Azevedo, 2006).

2.3 Barley proteins

Barley proteins are a major source of nutrition for humans and livestock and account for about 10% of the dry weight of mature barley grains. T.B. Osborne classified seed proteins into three groups according to their function namely, storage, structural and metabolic and protective proteins. Storage proteins accumulate in the seed and provide a reserve for the developing seedling; they also have distinct nutritional and physical properties (Halford, 1999). Storage proteins determine not only the total protein content of seed but its quality influences the use of grains in food processing, for example, wheat for bread and barley malt for beer (Shewry *et al*., 1995). Osborne further classified storage proteins into four fractions based on their solubility namely, albumins (soluble in water), globulins (soluble in dilute salt) (Shewry and Halford, 2002), prolamins (soluble in alcohol) (Howard *et al*., 1996) and glutelins (soluble in sodium dodecyl sulphate) (Celus *et al*., 2006).

Prolamins, also called hordeins, (in barley) are the major storage proteins (about 20 - 30 fractions), which account for 35 - 50% of the total grain N depending on the cultivar (Howard *et al*., 1996) and the amount of N fertilizer applied (Shrewry, 1992; Brennan *et al*., 1996). There are four types of hordeins which are classified by their amino acid composition and sequences: main types are B hordein (sulphur rich) and C hordein (sulphur poor), which comprise 70 - 80% and 10 - 20% fractions respectively and minor types are γ (sulphur rich) and D hordein (high molecular weight), which comprise less than 5% of the total hordein fraction. Hordeins have been used for cultivar identification but their roles within the matrix and relationship to malting quality is unknown (Shewry and Tatham, 1990; Howard *et al*., 1996).

Hordeins are synthesised on the rough endoplasmic reticulum and accumulate in protein bodies (Mundy *et al*., 1986) during mid-to late grain filling period. They are ruptured to form a protein matrix that surrounds the starch granules within the endosperm cells. The degradation of hordeins is necessary for two reasons, firstly during germination to support the growing embryo and similarly during malting to provide passage to starch degrading enzymes to the starch for complete starch hydrolysis (Howard *et al*., 1996).

CHAPTER 3

The use of leaf nitrogen to determine kernel nitrogen of a doubled haploid population of malting barley under irrigation

3.1 Introduction

Nitrogen (N) is one of the most limiting nutrients in most of the world's crops, therefore sufficient N in the form of fertilizer must be applied. However, one of the requirements for good malting quality is barley kernels with an N content of 1.5 - 1.95% (De Ruiter, 1999). A good strategy to improve the N content has been to apply split applications of N at different stages of plant growth, this way enough N will be available for efficient plant growth to obtain good yield and limited N will be found in the barley kernels to ensure good malting quality (Baethgen *et al*., 1995).

In previous studies, N status of a malting barley crop was assessed with tissue sampling and/or with the use of chlorophyll meters. However, tissue sampling is time consuming and expensive and chlorophyll meters cannot indicate how much excessive N is available to the crop (Wright *et al*., 2004; Francis and Piekielek, 2007). The approach in this study was to study the relationship between N content in leaves and N content in mature barley kernels. Nitrogen fertilizer was applied at different stages of plant growth development and N content in leaf samples was measured approximately four weeks after the N fertilizer treatment to determine the effect of the treatment. The N content in leaves could be a guide on how much N should be applied at the different plant growth stages during crop development in an attempt to manipulate the N content in the mature kernels. Thus the N content in leaves is the driving factor for scheduling N application to get optimum N content in kernels to obtain good malting quality.

The main objective of this study was thus to use the leaf N to predict the kernel N and use the information to implement a practical N fertilizer management system to obtain good malting quality.

3.2 Materials and methods

3.2.1 Materials

A barley doubled haploid population consisting of 74 lines was used in this study. This population was developed by crossing a two-row barley, Extract (developed by the University of Minnesota, USA) which has high malt extract yield and good malt quality with a six-row barley, Excel (developed by the Minnesota Agricultural Experiment Station, USA) which has good disease resistance and high yield. The F1 generation consisted of six-row progeny. This progeny was used to produce the doubled haploid lines and during this process progeny consisting of both two-row (7 lines), six-row (67 lines) and mixed (2 lines) populations were produced due to genetic instability. Mixed progeny (that is, progeny which consisted of both two- and six-row spikes on one plant) were discarded due to it being mixed and due to the small number. The doubled haploid lines were developed at the ARC-Small Grain Institute in Bethlehem with the anther culture technique (V. Daniel, Bayer Landesanstalt für Bodenkultur und Pflanzenbau in Freising, Germany, personal communication, 2000). The parents and progeny were planted under irrigation at Vaalharts Research Station in the Northern Cape in 2006 and 2007 as well as the Rietriver Research Station in the Northern Cape in 2007. These research stations are classified as the cooler irrigation areas of South Africa.

Four identical plots were planted for the four different N fertilizer treatments. For each plot, twenty plants were planted per entry 10 cm apart in a row (2 m). A total of 110 kg/ha fertilizer was applied per treatment consisting of 45 kg nitrogen (N), 30 kg phosphorous (P) and 35 kg potassium (K) (7:2:3 (31) + 0.5%) at different plant growth development stages. For treatment one, all of the fertilizer (110 kg/ha) was applied at planting. For treatments two, three and four, half of the fertilizer (55 kg/ha) was applied at planting while the other half (55 kg/ha) was applied at the six-leaf stage, when 50% of flag leaves were visible and when 50% spikes were visible respectively. Leaf samples (the uppermost leaf of the plant) were collected per entry from all the treatment plots four weeks after each treatment was applied irrespective whether all the N had been applied to that plot or not. It was not possible to collect leaf samples for treatment four due to heavy rains and for this reason treatment four was omitted from this chapter. Kernels per entry for each treatment were collected at harvesting. All plant material was harvested after each season.

3.2.2 Methods

3.2.2.1 Total nitrogen

The Leco® FP-2000 Nitrogen/Protein Analyzer was used to determine the total N content of the leaf and kernel samples collected. Leaf and kernel samples were dried in a 50ºC dry-oven overnight and soil samples were air-dried and sieved through a 0.5 µm sieve. The samples were weighed and loaded into ceramic boats and placed into the furnace at 1050ºC. Oxygen (O_2) flows into the furnace and the samples combust to form nitrogen (N_2), nitrous oxide (NO_x), carbon dioxide (CO_2), water (H_2O), and O_2. These gasses collect in the ballast tank and are equilibrated under high pressure. Only 10cc aliquots of the combustion products are passed over hot copper to remove O_2 and convert NO_X to N_2. Lecosorb removes CO_2 and anhydrone removes H_2O and the helium gas is used as the carrier for N_2, which is measured by the thermal conductivity detector and the result is expressed as percentage N (Anonymous, 1996; 2000). Protein content was calculated as N x 6.25% (Birch and Long, 1990).

3.2.2.2 Statistical analysis

Correlations were determined for the two- and six-row populations respectively over years (Vaalharts cropping season 2006 and 2007) as well as over localities (Vaalharts and Rietriver cropping season 2007) and for each of the three N treatments. The leaf and kernel N content (%) for each doubled haploid line per population was added and the average was used for the correlation analysis. Statistical analysis was done using Number Cruncher Statistical System (NCSS) (Hintze, 2004). Replicate testing within environments/localities was impossible because of the cost and time for sampling and analysis of the large doubled haploid population used in this study.

3.3 Results

3.3.1 Effect of timing of nitrogen fertilizer application on two- and six-row populations

3.3.1.1 Treatments over years

In the two-row population there were no significant correlations between leaf and kernel N for treatment one across all treatment plots at Vaalharts in 2006 and 2007 (Table 3.1). However, there were significant correlations ($P \leq 0.01$) between leaf and kernel N in the

six-row population for treatment one across all treatment plots i.e. when all the fertilizer was applied at planting at treatment plot one and half of the fertilizer was applied at planting at treatment plots two and three.

Table 3.1. Correlations between leaf and kernel N over years for treatment one

Location	Vaalharts 2006 and 2007		
Treatment plot	1	2	3
Two-row population	-0.19^{ns}	0.27^{ns}	-0.34^{ns}
Six-row population	**0.26****	**0.60****	**0.36****

**** p≤0.01**, ^{ns} not significant

There were no significant correlations between leaf and kernel N in the two-row population for treatment two across all treatment plots over years (Table 3.2). In the six-row population a significant relationship (P ≤ 0.05) existed between kernel and leaf N for treatment two only at treatment plot three were only half of the fertilizer was applied at planting.

Table 3.2. Correlations between leaf and kernel N over years for treatment two

Location	Vaalharts 2006 and 2007		
Treatment plot	1	2	3
Two-row population	-0.21^{ns}	0.07^{ns}	0.52^{ns}
Six-row population	-0.00^{ns}	0.01^{ns}	**0.20***

*** p≤0.05**, ^{ns} not significant

A significant correlation (P ≤ 0.05) between leaf and kernel N occurred in the two-row population for treatment three at treatment plot three only when half the fertilizer was applied at planting and half at flag leaf stage (Table 3.3). There were no significant correlations between leaf and kernel N in the six-row population for treatment three across all treatment plots over years.

Table 3.3. Correlations between leaf and kernel N over years for treatment three

Location	Vaalharts 2006 and 2007		
Treatment plot	1	2	3
Two-row population	-0.01^{ns}	0.31^{ns}	**0.54***
Six-row population	0.03^{ns}	-0.02^{ns}	0.13^{ns}

*** p≤0.05**, ^{ns} not significant

3.3.1.2 Treatments over localities

There were no significant correlations for leaf and kernel N for two- and six-row populations found for treatments one and two across all treatment plots at Vaalharts and Rietriver in 2007 (Table 3.4 and 3.5).

Table 3.4. Correlations between leaf and kernel N over localities for treatment one

Location	Vaalharts and Rietriver 2007		
Treatment plot	1	2	3
Two-row population	-0.35^{ns}	0.30^{ns}	-0.35^{ns}
Six-row population	0.03^{ns}	0.16^{ns}	-0.06^{ns}

^{ns} not significant

Table 3.5. Correlations between leaf and kernel N over localities for treatment two

Location	Vaalharts and Rietriver 2007		
Treatment plot	1	2	3
Two-row population	-0.01^{ns}	0.45^{ns}	0.53^{ns}
Six-row population	0.15^{ns}	-0.01^{ns}	0.18^{ns}

^{ns} not significant

In the two-row population there was a significant relationship ($P \leq 0.05$) between leaf and kernel N for treatment three at treatment plot three only when half the fertilizer was applied at planting and half at flag leaf stage (Table 3.6). However, in the six-row population there were significant correlations ($P \leq 0.01$) for treatment three at treatment plots two and three when half the fertilizer was applied at planting and half at the six-leaf and flag leaf stage respectively.

Table 3.6. Correlations between leaf and kernel N over localities for treatment three

Location	Vaalharts and Rietriver 2007		
Treatment plot	1	2	3
Two-row population	-0.01^{ns}	0.31^{ns}	**0.54***
Six-row population	0.08^{ns}	**0.51****	**0.24****

**** p≤0.01, * p≤0.05**, ^{ns} not significant

3.3.2 Total nitrogen

Although there were significant correlations for two- and six-row populations for some treatments over years and localities, N content in kernels were not within the acceptable range of 1.5 - 1.95% for good barley malting quality. Kernel N content was within the accepted specification only at Rietriver in 2007 for treatment two for both the two- and six-row populations (1.61 and 1.91% respectively, Table 3.7).

Table 3.7. Average kernel N% for two- and six-row populations over years and localities

Location	Vaalharts 2006			Vaalharts 2007			Rietriver 2007		
Treatment	1	2	3	1	2	3	1	**2**	3
Two-row population	2.30	2.24	2.11	2.64	2.60	2.74	2.12	**1.61**	2.16
Six-row population	2.72	2.57	2.49	2.92	2.88	2.73	2.05	**1.91**	2.17

Note: Values in **bold** were within the acceptable range of 1.5 - 1.95% for kernel N

3.3.3 Effect of timing of nitrogen fertilizer application on single plants in a population

Only the significant correlations between leaf and kernel N at the different N treatments for both two- and six-row entries reported in section 3.3.1 were used to determine the effect of timing of N fertilizer application on single plants. Histograms were used to determine whether leaf and kernel N for the two- and six-row entries responded to the N treatments consistently over years and environments. Figures 3.1 - 3.7 show that the single entries within a population for both two- and six-row populations varied in

response to the different N treatments over years and localities. For example in Figure 3.1, leaf N% of two-row entry 12 ranked 2nd at Vaalharts in 2006, 5th at Vaalharts in 2007 and 4th at Rietriver in 2007 and for kernel N% entry 12 ranked 4th at Vaalharts in 2006, 5th at Vaalharts in 2007 and 6th at Rietriver in 2007. The 12 six-row entries shown in Figures 3.2 - 3.7 represented the six-row population as all 67 entries varied in response to N treatments over years and localities.

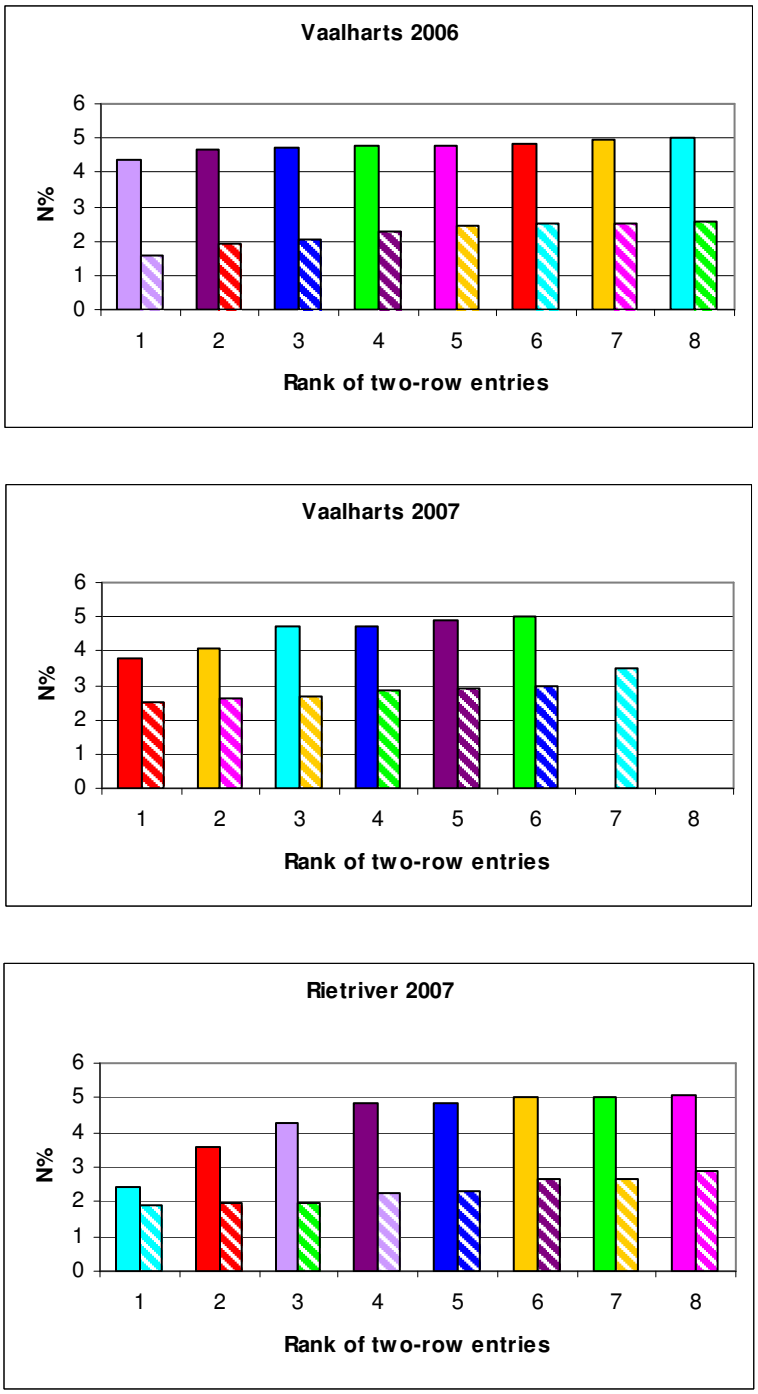

Figure 3.1. Ranking of two-row entries for leaf and kernel N (%) over years and localities at treatment three, treatment plot three (where leaf N% is represented as solid bars and kernel N% as patterned bars. Two-row entries are colour coded as red = entry 1, ceres = entry 7, purple = entry 12, yellow = entry 34, green = entry 56, royal blue = entry 67, turquoise = entry 68 and lilac = entry 78).

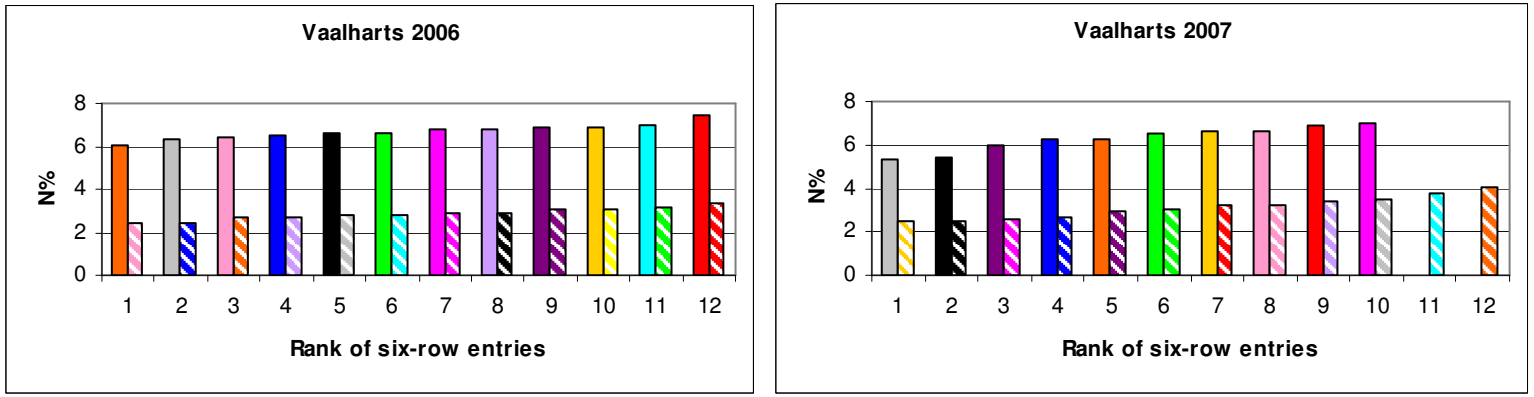

Figure 3.2. Ranking of six-row entries for leaf and kernel N (%) over years at treatment one, treatment plot one

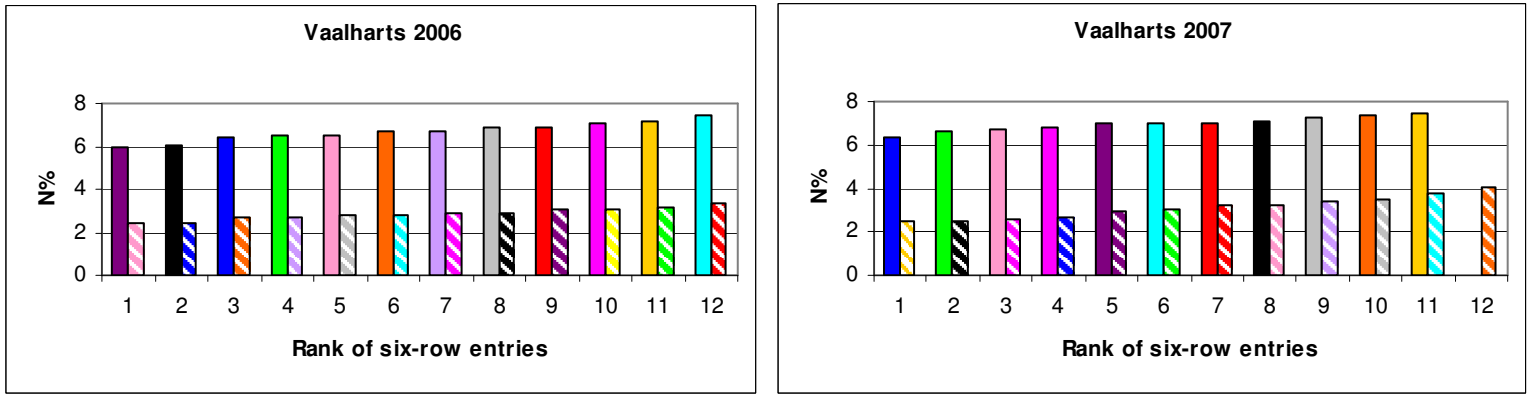

Figure 3.3. Ranking of six-row entries for leaf and kernel N (%) over years at treatment one, treatment plot two (where leaf N% is represented as solid bars and kernel N% as patterned bars. Six-row entries are colour coded as red = entry 3, ceres = entry 4, purple = entry 5, yellow = entry 6, green = entry 8, royal blue = entry 10, turquoise = entry 11, lilac = entry 13, black = entry 14, pink = entry 15, orange = entry 16 and grey = entry 17).

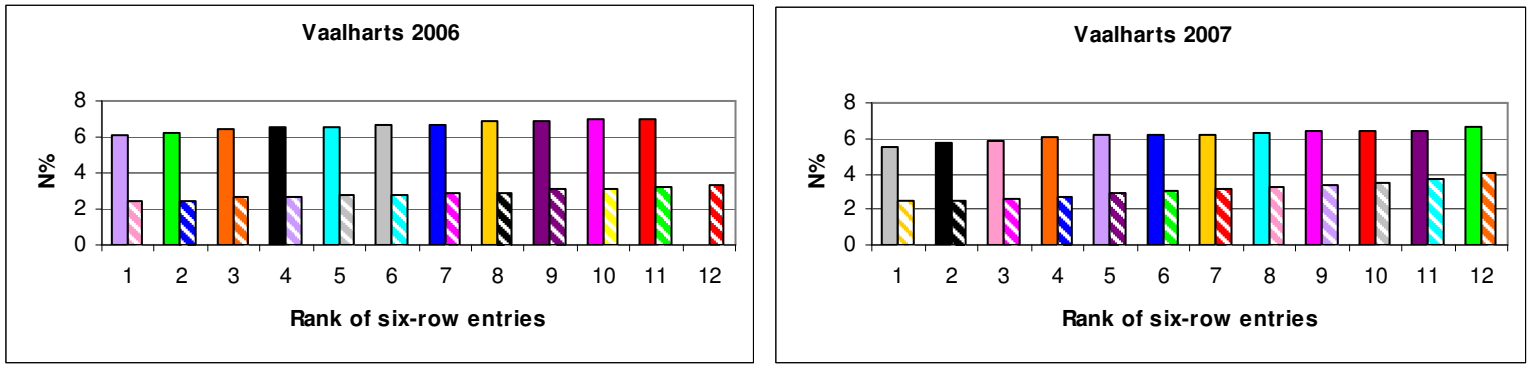

Figure 3.4. Ranking of six-row entries for leaf and kernel N (%) over years at treatment one, treatment plot three

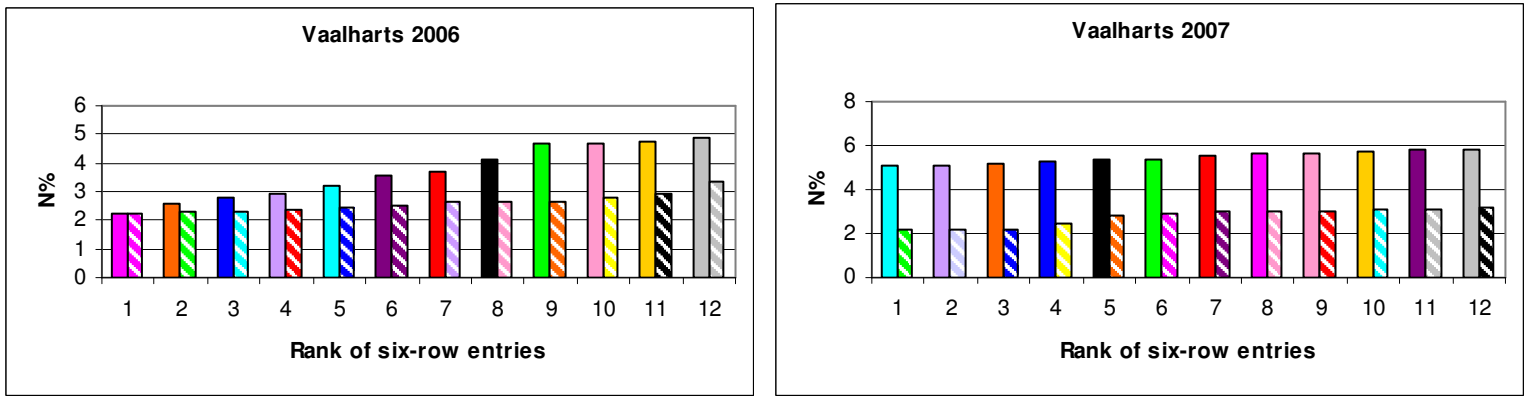

Figure 3.5. Ranking of six-row entries for leaf and kernel N (%) over years at treatment two, treatment plot three (where leaf N% is represented as solid bars and kernel N% as patterned bars. Six-row entries are colour coded as red = entry 3, ceres = entry 4, purple = entry 5, yellow = entry 6, green = entry 8, royal blue = entry 10, turquoise = entry 11, lilac = entry 13, black = entry 14, pink = entry 15, orange = entry 16 and grey = entry 17).

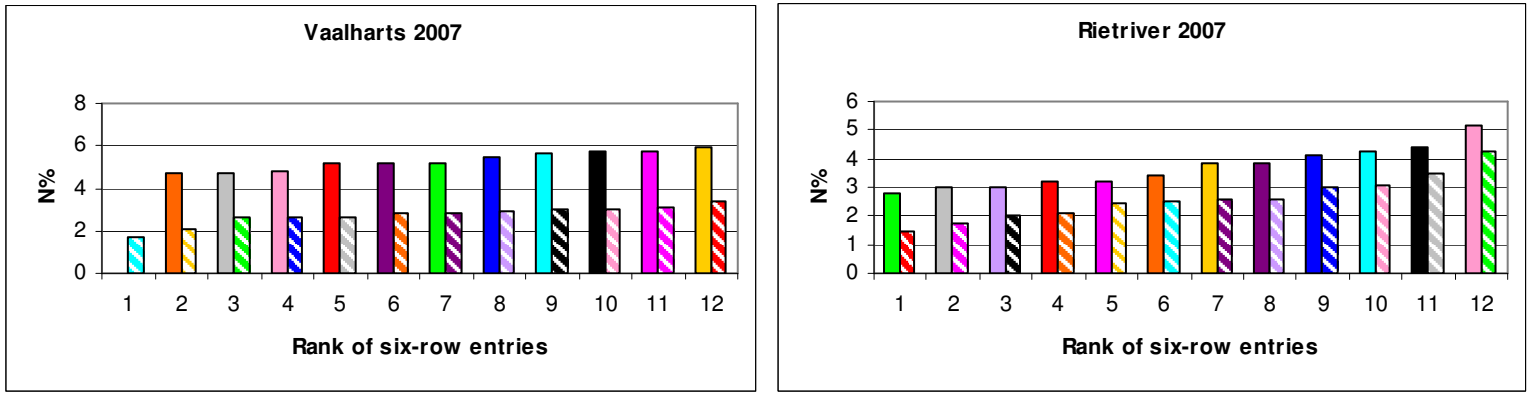

Figure 3.6. Ranking of six-row entries for leaf and kernel N (%) over localities at treatment three, treatment plot two

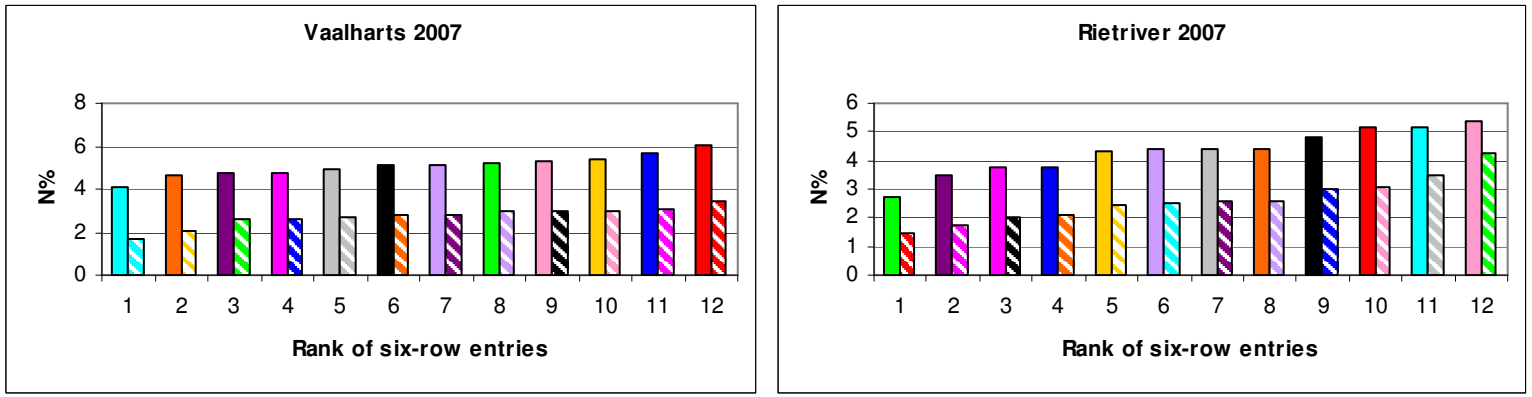

Figure 3.7. Ranking of six-row entries for leaf and kernel N (%) over localities at treatment three, treatment plot three (where leaf N% is represented as solid bars and kernel N% as patterned bars. Six-row entries are colour coded as red = entry 3, ceres = entry 4, purple = entry 5, yellow = entry 6, green = entry 8, royal blue = entry 10, turquoise = entry 11, lilac = entry 13, black = entry 14, pink = entry 15, orange = entry 16 and grey = entry 17).

3.4 Discussion

The two- and six-row populations responded completely different to N fertilizer application at the different plant growth development stages. The best application for the two-row population over years and localities was when N was applied half at planting and half at flag leaf stage (Table 3.3 and 3.6 respectively). However, N content in kernels were not within the acceptable range (1.5 - 1.95%). As a result, different rates of N fertilizer application have to be tested. Only eight entries made up the two-row population. It is not known if a larger population will respond differently to the N treatments.

The six-row population responded differently to N application over years and localities. Over years the best application of N was at treatment one for all treatment plots (Table 3.1) i.e. when all N was applied at planting and also when only half the N was applied at planting and at treatment two for treatment plot three (Table 3.2) i.e. when half the N was applied at planting only. Over localities the best application of N was at treatment three at treatment plots two and three (Table 3.6), when half the N was applied at planting and the other half was applied at six-leaf and flag leaf stage respectively. From the results it can be seen that the best application for the six-row population may be to apply N fertilizer half at planting and the other half as a split application at the six-leaf and flag leaf stages. Kernel N content was within the required specification (1.61 and 1.91%) for two- and six-row populations respectively only at Rietriver in 2007. This indicates that the environment should be taken into consideration when implementing fertilizer management systems. Various studies have shown that kernel protein content is mainly controlled by genetic factors but is also largely influenced by the environment (Chen *et al*., 2006).

Two- and six row populations responded differently in terms of N uptake and translocation to the kernels. Due to the differences in the size and amount of kernels needed to be filled in a two-row spike compared to a six-row spike, protein variation may by more evident in a six-row plant than a two-row plant (Ellis and Marshall, 1998). Therefore the response of different cultivars to N application will also have to be determined.

The best N application for the two- and six-row populations were determined based on an average of the individual lines making up each population. However, the single entries per population responded differently over years and localities for both populations. It is therefore, impossible to sample leaves randomly from a population in an attempt to decide whether to apply N at a particular plant growth stage to obtain optimum N content in mature kernels, due to genetic variation and environmental influence. Correlations were significant however, they were generally low and explained little of the variation that occurred between leaf and kernel N.

CHAPTER 4

The influence of nitrogen fertilizer application on malting quality of irrigation barley

4.1 Introduction

Kernel plumpness, germination and kernel protein content are important characteristics that determine malting quality (Mather *et al*., 1997). These parameters must be consistent in a range of environments to produce grain quality that is acceptable for the malting industry (Marshall and Ellis, 1998). Superior grain quality and high yield may be achieved by appropriate nitrogen fertilizer and irrigation practices in the field (De Ruiter, 1999).

The rate and timing of nitrogen (N) applications is crucial for optimum grain quality and yield. Insufficient N may reduce yield and quality while excessive N increases yield and kernel protein content which is unacceptable, as malting quality is reduced, excessive N also decreases kernel plumpness. However, high fertilizer applications may also result in a decrease in yield, as lodging may occur (Lauer and Partridge, 1990; Thompson *et al*., 2004).

Various studies have shown that fertilizer should be applied at sowing to encourage crop and tiller development and at the end of tillering to enhance the yield. Application of N during stem extension may result in increased yield but also high kernel N content. Therefore, interaction of N fertilizer application with malting quality is very complex (Chen *et al*., 2006). However, a split N application at tillering and boot (swelling of flag leaf sheath - Figure 4.1) stages resulted in better N content whilst a single N application at tillering enhanced yield (Baethgen *et al*., 1995; Sardana and Zhang, 2005a; b).

The purpose of this study was to investigate the influence of N fertilizer application at different stages of plant growth development on malting quality characteristics of two- and six-row doubled haploid populations.

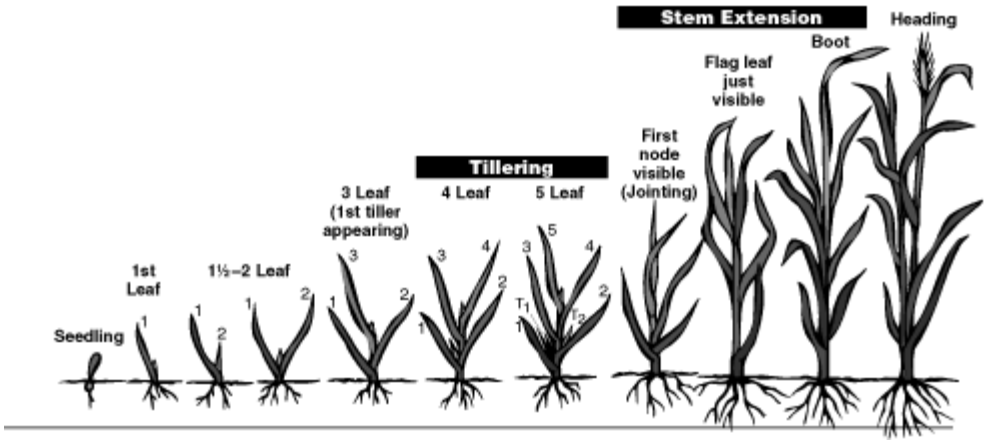

Figure 4.1. Growth stages of barley crop (Gregoire *et al*., 2007)

4.2 Materials and methods

4.2.1 Materials

The same plant material, fertilizer rate, treatments and localities over years as described in Chapter 3 were employed to investigate the influence of N treatments at different stages of plant growth on malting quality. For this study, four identical plots were sampled for four different N fertilizer treatments in contrast to the three of the previous chapter. For each plot, twenty plants were planted per entry 10 cm apart in a row (2 m).

4.2.2 Methods

4.2.2.1 Kernel plumpness

The 20 single plants per entry per row for each N treatment were pooled and the total kernel weight was determined. The seeds (100 g) were separated with a Sortimat falling number AB sieve shaker (Stockholm, Sweden) for 1 min according to 2.8 mm, 2.5 mm and 2.2 mm fractions. Kernel plumpness percentage was determined by adding the 2.8 mm and 2.5 mm fractions percentages. The yield (t/ha) was calculated with the total kernel weight (g) for all 20 plants per entry per row for each N treatment. Before yield could be determined a conversion factor (that is, to convert gram per plot to ton per hectare) for all plots had to be calculated.

4.2.2.2 Germination

Germination rate was determined by placing 10 seeds per entry in a petri dish containing 8 ml of distilled water. These were incubated at 20ºC and allowed to germinate at the following times: 24, 48 and 72 h. Complete germination at 48 h is desirable, however complete germination after 72 h is acceptable. These tests were repeated three times for each N treatment at two week intervals to determine whether there were any dormancy effects (Anonymous, 2007).

4.2.2.3 Absorption test

Absorption rate was determined by placing 10 seeds per entry in boiling water for 2 min. The seeds were dried with paper towel and cut in half with the vitreous kernel instrument to determine the water penetration ability of each seed. This was measured by observing a gel-like appearance within the seed and was rated as follows: no gel-like appearance scores 0, ¼ scores 1, ¾ scores 3 and complete absorption scores 4. A percentage of the scores for 10 seeds were taken and the average was used to determine the absorption rate per entry for each N treatment.

4.2.2.4 Total nitrogen

As explained in Chapter 3 (section *3.2.2.1*).

4.2.2.5 Statistical analyses

The interactions between the different malting quality traits were determined by using simple linear correlations for the two- and six-row population respectively over years (Vaalharts in 2006 and 2007) and over localities (Vaalharts and Rietriver in 2007) and for each of the four N treatments. Stepwise regression was also used to determine which malting traits contributed most to the variation in a particular trait. This was determined for both the two- and six-row populations respectively and the analysis was combined over both localities and years for each N treatment. Statistical analyses were done using Number Cruncher Statistical System (NCSS) (Hintze, 2004). Analysis of variance (ANOVA) was carried out to determine the effect of the environment over years on the different genotypes by using Agrobase® (Mulitze, 2000).

4.3 Results

4.3.1 Malting quality

4.3.1.1 Kernel plumpness

The best N application for kernel plumpness for the two-row population was treatment two when half the N was applied at planting and half at six-leaf stage (Table 4.1). However, kernel plumpness was only in the required specification (i.e. >80%) for two-row barley at Vaalharts in 2006 and Rietriver in 2007. The six-row population responded differently to N treatments over the three localities and was below the required specification (i.e. >70%) for kernel plumpness.

Table 4.1. Average kernel plumpness (%) for two- and six-row populations for all environments

Location	Vaalharts 2006				Vaalharts 2007				Rietriver 2007			
Treatment	1	2	3	4	1	2	3	4	1	2	3	4
Two-row population	82.46	**83.33**	71.24	80.17	57.35	**60.19**	52.55	53.64	79.52	**88.37**	64.12	66.56
Six-row population	49.57	50.94	**52.15**	51.39	**58.96**	54.58	54.99	53.49	36.69	**38.01**	33.47	23.43

Note: Values in **bold** show which N application resulted in the highest kernel plumpness (%)

4.3.1.2 Yield

The highest yields for the two- and six-row population, 8.08 and 4.97 t/ha respectively, were obtained at Vaalharts in 2006 with treatment four, when half the N was applied at planting and half the N when 50% spikes were visible (Table 4.2). However, this application produced the lowest yield in the next season. Yield for the two- and six-row populations responded differently to N application at Vaalharts and Rietriver in 2007. Vaalharts 2007, treatment three (half the N at planting and half at flag leaf stage) produced the highest yield for the two-row population and treatment two (half the N at planting and half at six-leaf stage) for the six-row population. Treatment two produced the best yield for the two-row population and treatment one (all the N at planting) for the six-row population at Rietriver in 2007. Yield for all N treatments at Rietriver in 2007 was low compared to Vaalharts in 2006 and 2007.

Table 4.2. Average yield (t/ha) for two- and six-row populations for all environments

Location	Vaalharts 2006				Vaalharts 2007				Rietriver 2007			
Treatment	1	2	3	4	1	2	3	4	1	2	3	4
Two-row population	4.83	6.90	6.37	**8.08**	5.62	5.93	**6.46**	4.52	2.68	**2.90**	2.09	1.67
Six-row population	4.13	4.74	4.96	**4.97**	5.39	**5.94**	5.79	5.38	**1.26**	1.21	1.18	0.79

Note: Values in **bold** show which N application resulted in the highest yield (t/ha)

4.3.1.3 Germination

The N application that influenced germination the most for the two-row population was treatment two, when half the N was applied at planting and half the N at six-leaf stage at Vaalharts in 2006 and 2007 (Tables 4.3 - 4.4). However, treatment three (i.e. when half the N was applied at planting and half at flag leaf stage) gave the best results at Rietriver in 2007 (Table 4.5). For the six-row population, the best N application was treatment three for all environments. However at Rietriver in 2007, treatment one where all the N was applied at planting also gave an excellent germination response. Germination rate was higher in 2007 compared to 2006.

Table 4.3. Average germination (%) for two- and six-row populations at Vaalharts 2006

Treatment	1			2			3			4		
Time (h)	24	48	72	24	48	72	24	48	72	24	48	72
Two-row population	50.0	60.9	67.6	**57.6**	**65.7**	**71.4**	53.8	63.3	71.0	51.4	60.0	71.4
Six-row population	54.9	59.1	63.9	59.1	63.2	68.5	**62.7**	**65.8**	**69.0**	61.7	64.3	67.6

Note: Values in **bold** show which N application resulted in the highest germination rate (%)

Table 4.4. Average germination (%) for two- and six-row populations at Vaalharts 2007

Treatment	1			2			3			4		
Time (h)	24	48	72	24	48	72	24	48	72	24	48	72
Two-row population	82.2	84.4	86.6	**86.7**	**86.7**	**91.1**	78.9	80.0	83.9	77.2	78.3	79.4
Six-row population	85.2	82.6	85.7	81.0	82.7	85.3	**84.2**	**85.7**	**87.2**	83.5	84.9	86.1

Note: Values in **bold** show which N application resulted in the highest germination rate (%)

Table 4.5. Average germination (%) for two- and six-row populations at Rietriver 2007

Treatment	1			2			3			4		
Time (h)	24	48	72	24	48	72	24	48	72	24	48	72
Two-row population	71.5	78.1	80.0	71.9	81.9	83.3	**79.5**	**88.1**	**90.5**	67.2	82.4	82.8
Six-row population	**84.4**	**85.4**	**85.5**	83.1	84.8	85.4	**83.2**	**85.0**	**85.8**	80.2	82.4	82.9

Note: Values in **bold** show which N application resulted in the highest germination rate (%)

4.3.1.4 Absorption

Absorption rate for both populations responded differently to N application over all environments. The best N application for absorption was at Vaalharts in 2007 at treatment three when half the N was applied at planting and half at flag leaf stage for the two-row population and at treatment two when half the N was applied at planting and half at six-leaf stage for the six-row population (Table 4.6).

Table 4.6. Average absorption rate (%) for two- and six-row populations for all environments

Location	Vaalharts 2006				Vaalharts 2007				Rietriver 2007			
Treatment	1	2	3	4	1	2	3	4	1	2	3	4
Two-row population	59.3	49.3	59.0	**60.3**	73.5	63.3	**74.3**	67.0	47.8	46.5	**52.8**	44.0
Six-row population	56.5	55.0	**61.3**	59.0	69.5	**73.3**	69.3	69.3	37.0	38.5	**42.8**	39.3

Note: Values in **bold** show which N application resulted in the highest absorption rate (%)

4.3.1.5 Total nitrogen

Total N content in kernels were not within the accepted specification (1.5 - 1.95%) for good barley malting quality for both populations at Vaalharts in 2006 and 2007 (Table 4.7). However, kernel N was within the required specification at treatment two when half the N was applied at planting and half at six-leaf stage for both populations and for the two-row population at treatment four when half the N was applied at planting and half when 50% spikes were visible at Rietriver in 2007. Treatment three resulted in lower kernel N content compared to the other N treatments at Vaalharts in 2006 but this did not occur in the following season.

Table 4.7. Average kernel N (%) for two- and six-row populations for all environments

Location	Vaalharts 2006				Vaalharts 2007				Rietriver 2007			
Treatment	1	2	3	4	1	2	3	4	1	2	3	4
Two-row population	2.30	2.24	2.11	2.24	2.64	2.60	2.74	2.64	2.12	**1.61**	2.16	**1.94**
Six-row population	2.72	2.57	2.49	2.50	2.92	2.88	2.73	2.85	2.05	**1.91**	2.17	2.35

Note: Values in **bold** were within the acceptable range of 1.5 - 1.95% for kernel N

4.3.2 Simple ANOVA analysis over years

4.3.2.1 Kernel plumpness

The CV of 36.98% is unacceptable for irrigation barley trials planted at one locality over two years. This is due to the fact that localities were used as the replication component in the analysis. There were no significant differences in kernel plumpness between the four treatments, between treatments over years and for entries between treatments over the two years at Vaalharts (Table 4.8). There were significant differences between entries. Therefore, it was possible to compare the entries by their means. Genotype sum of squares contributed 80.1% of the total sum of squares among the entries while 19.9% variation was due to the environment.

4.3.2.2 Yield

The CV of 58.30% is very high. There were no significant differences in yield between the four treatments, between treatments over years and for entries between treatments

over the two years at Vaalharts (Table 4.8). However significant differences were found between entries. Genotypes contributed 61.9% of the total variation among the entries compared to the environment (38.1%).

4.3.2.3 Kernel nitrogen

The CV of 14.35% was acceptable for irrigation barley trials planted at one locality over two years. There were significant differences in kernel N between the four treatments and between treatments over the two years at Vaalharts (Table 4.8). There were significant differences between entries. Therefore, it was possible to compare the entries by their means. There were no significant differences in kernel N for entries between treatments. Genotypes contributed 40.3% of the total variation among the entries while 59.7% was due to environmental influence.

4.3.2.4 Kernel protein

The CV of 14.07% is acceptable. There were significant differences in kernel protein N between the four treatments and between treatments over the two years at Vaalharts (Table 4.8). There were significant differences between entries. There were no significant differences in kernel protein for entries between treatments. For kernel N the environment (56.6%) contributed more to the total variation among the entries compared to the genotype (43.4%).

Table 4.8. Simple ANOVA over years for malting quality traits

Source	Kernel plumpness	Yield	Kernel N	Kernel protein
Treatment	67.43^{ns}	14.49^{ns}	**1.06****	**37.51****
Year in treatment	334.98^{ns}	17.42^{ns}	**2.60****	**105.44****
Entry	**1179.01****	**19.38****	**0.20***	**8.07***
Entry x treatment	234.94^{ns}	7.39^{ns}	0.12^{ns}	4.57^{ns}
LSD for entry	16.81	2.51	0.32	1.95
Heritability	0.80	0.62	0.40	0.43

**** p≤0.01, * p≤0.05**, ^{ns} not significant

4.3.3 Simple ANOVA over localities

4.3.3.1 Kernel plumpness

The CV of 47.97% is very high. There were significant differences in kernel plumpness between the four treatments, between treatments over the two localities and between entries (Table 4.9). There were no significant differences for entries between treatments. Genotypes contributed 73.9% of the total variation among the entries while 26.1% was due to environmental influence.

4.3.3.2 Yield

The CV of 80.87% is unacceptable for irrigation barley trials planted at one locality over two years, and given this high percentage, this data cannot be accurately interpreted.

4.3.3.3 Kernel nitrogen

The CV of 22.13% is relatively high for irrigation barley trials planted at one locality over two years. There were no significant differences in seed N between the four treatments, between entries and for entries between treatments over both localities in 2007 (Table 4.9). However there were significant differences for treatments over the two localities. Genotypes contributed 14.8% of the total variation among the entries. Therefore, there was 85.2% environmental influence.

4.3.3.4 Kernel protein

The CV of 21.98% is relatively high. There were no significant differences in kernel protein N between the four treatments, between entries and for entries between treatments over both localities in 2007 (Table 4.9). However there were significant differences for treatments over the two localities. Genotypes contributed 13.2% of the total variation among the entries. Therefore, there was 86.8% environmental influence.

Table 4.9. Simple ANOVA over localities for malting quality traits

Source	Kernel plumpness	Yield	Kernel N	Kernel protein
Treatment	**2544.28****	8.39^{ns}	0.12^{ns}	4.06^{ns}
Year in treatment	**12589.28****	**676.05****	**19.31****	**765.35****
Entry	**1269.03****	**13.97****	0.31^{ns}	11.63^{ns}
Entry x treatment	330.68^{ns}	5.70^{ns}	0.26^{ns}	10.09^{ns}
LSD for entry	18.59	2.24	0.45	2.80
Heritability	0.74	0.59	0.15	0.13

**** p≤0.01**, ^{ns} not significant

4.3.4 Stepwise regression for the two-row population across all environments

4.3.4.1 Kernel plumpness

The different malting traits did not contribute significantly to kernel plumpness variation for treatments one and four for the two-row population (Table 4.10). For treatment two, kernel plumpness was explained by yield (9%) however, this relationship was not significant but for treatment three, yield contributed 20% to kernel plumpness at P ≤ 0.01.

4.3.4.2 Kernel protein

For treatment one, kernel protein variation was explained by absorbance (20%) and kernel plumpness (13%) (Table 4.10). Absorbance and kernel plumpness were significant at P ≤ 0.01. For treatment three, only absorbance (27%) contributed to kernel protein variation at P ≤ 0.01. No malting traits contributed to kernel protein variation for treatments two and four for the two-row population.

Table 4.10. Regression analysis for the two-row population at all N treatments across environments

Treatment	Malting quality trait 1	Malting quality trait 2	R-squared value	Contribution of each trait to total variation (%)
1	Kernel plumpness	-	-	-
2	Kernel plumpness	Yield	0.09^{ns}	9
3	Kernel plumpness	Yield	**0.20****	**20**
4	Kernel plumpness	-	-	-
1	Kernel protein	Absorbance	**0.20****	**20**
		Kernel plumpness	**0.33****	**13**
2	Kernel protein	-	-	-
3	Kernel protein	Absorbance	**0.27****	**27**
4	Kernel protein	-	-	-

** **p≤0.01**, ^{ns} not significant

4.3.5 Stepwise regression for the six-row population across all environments

4.3.5.1 Kernel plumpness

Kernel plumpness variation was explained by yield for treatments one (35%), two (28%) and three (32%) at P ≤ 0.01 (Table 4.11). Absorbance (2%) also contributed to kernel plumpness variation however, this was not significant. Malting traits did not contribute to kernel plumpness variation for treatment four for the six-row population.

4.3.5.2 Kernel protein

For treatment one, kernel protein variation was explained by absorbance (27%), significant at P ≤ 0.01 (Table 4.11). For treatment two, yield (1%) contributed to seed protein variation however, this relationship was not significant. For treatments three and

four, 20% and 10% absorbance respectively contributed to seed protein variation with significant R-squared values at P ≤ 0.01.

Table 4.11. Regression analysis for the six-row population at all N treatments across environments

Treatment	Malting quality trait 1	Malting quality trait 2	R-squared value	Contribution of each trait to total variation (%)
1	Kernel plumpness	-	**0.35****	**35**
2	Kernel plumpness	Yield	**0.28****	**28**
3	Kernel plumpness	Yield	**0.32****	**32**
		Absorbance	0.34^{ns}	2
4	Kernel plumpness	-	-	-
1	Kernel protein	Absorbance	**0.27****	**27**
2	Kernel protein	Yield	0.01^{ns}	1
3	Kernel protein	Absorbance	**0.20****	**20**
4	Kernel protein	Absorbance	**0.10****	**10**

** **p≤0.01**, ^{ns} not significant

4.3.6 Linear correlations for two-row population over years

4.3.6.1 Kernel plumpness

A significant relationship existed between kernel plumpness and yield at treatments two and three (P ≤ 0.05) and treatment four (P ≤ 0.01) (Table 4.12). There was a significant relationship (P ≤ 0.05) between kernel plumpness and germination at 72 h for treatments one and two. There was a significant correlation (P ≤ 0.05) between kernel plumpness and kernel N at treatment two.

Table 4.12. Significant correlations between kernel plumpness and other malting quality traits for the two-row population at different N treatments over years

Treatment	Malting quality trait 1	Malting quality trait 2	Correlation value
2	Kernel plumpness	Yield	**0.62***
3	Kernel plumpness	Yield	**0.60***
4	Kernel plumpness	Yield	**0.77****
1	Kernel plumpness	Germination at 72 h	**0.58***
2	Kernel plumpness	Germination at 72 h	**0.53***
2	Kernel plumpness	Kernel N	**0.54***

**** p≤0.01, * p≤0.05**

4.3.6.2 Germination

There was a significant relationship between kernel N and germination at 24, 48 and 72 h for all treatments (Table 4.13). A significant relationship existed between absorption and germination at 24, 48, and 72 h at treatments one, three and four. There were significant correlations between all three germination tests at 24, 48 and 72 h for all treatments. There was a significant relationship between leaf N and germination at 24, 48 and 72 h at treatment three, treatment plot four (half the N applied at planting only).

Table 4.13. Significant correlations between germination and other malting quality traits for the two-row population at different N treatments over years

Treatment	Malting quality trait 1	Malting quality trait 2	Correlation value
1	Germination at 24, 48 and 72 h	Kernel N	**0.65*, 0.66* and 0.70****
2	Germination at 24, 48 and 72 h	Kernel N	**0.66*, 0.72** and 0.74****
3	Germination at 24, 48 and 72 h	Kernel N	**0.64*, 0.63* and 0.65***
4	Germination at 24, 48 and 72 h	Kernel N	**0.66*, 0.69** and 0.70****
1	Germination at 24, 48 and 72 h	Absorption	**0.67**, 0.76** and 0.76****
3	Germination at 24, 48 and 72 h	Absorption	**0.59*, 0.61* and 0.67****
4	Germination at 24, 48 and 72 h	Absorption	**0.61*, 0.63* and 0.69****
3	Germination at 24, 48 and 72 h	Leaf N (treatment plot four)	**0.61*, 0.60* and 0.68****

**** p≤0.01, * p≤0.05**

4.3.6.3 Absorption

A significant relationship (P ≤ 0.01) existed between kernel N and absorption at treatments one and four (Table 4.14).

Table 4.14. Significant correlations between absorption and other malting quality traits for the two-row population at different N treatments over years

Treatment	Malting quality trait 1	Malting quality trait 2	Correlation value
1	Absorption	Kernel N	**0.71****
4	Absorption	Kernel N	**0.75****

**** p≤0.01**

4.3.7 Linear correlations for two-row population over localities

4.3.7.1 Kernel plumpness

A significant relationship (P ≤ 0.05) existed between kernel plumpness and yield at treatment three (Table 4.15). There was a significant relationship between kernel plumpness and germination at 24, 48 and 72 h for treatments two and four. There was a significant negative correlation (P ≤ 0.05) between leaf N and kernel plumpness at treatment two, treatment plot two (half the N at planting and half at six-leaf stage).

Table 4.15. Significant correlations between kernel plumpness and other malting quality traits for the two-row population at different N treatments over localities

Treatment	Malting quality trait 1	Malting quality trait 2	Correlation value
3	Kernel plumpness	Yield	**0.60***
2	Kernel plumpness	Germination at 24, 48 and 72 h	**0.57*, 0.72** and 0.77****
4	Kernel plumpness	Germination at 24, 48 and 72 h	**0.55*, 0.59* and 0.54***
2	Kernel plumpness	Leaf N (treatment plot two)	**-0.54***

**** p≤0.01, * p≤0.05**

4.3.7.2 Germination

There was a significant relationship between kernel N and germination at 24, 48 and 72 h for all treatments (Table 4.16). There were significant correlations between all three germination tests at 24, 48 and 72 h for all treatments. A significant relationship existed between absorption and germination at 24, 48, and 72 h at treatments one, two and three. There was a significant relationship between leaf N and germination at 24, 48 and 72 h at treatment three, treatment plot four (half the N at planting only).

Table 4.16. Significant correlations between germination and other malting quality traits for the two-row population at different N treatments over localities

Treatment	Malting quality trait 1	Malting quality trait 2	Correlation value
1	Germination at 24, 48 and 72 h	Kernel N	**0.63**, 0.60* and 0.63****
2	Germination at 24, 48 and 72 h	Kernel N	**0.74**, 0.77** and 0.72****
3	Germination at 24, 48 and 72 h	Kernel N	**0.64**, 0.68** and 0.71****
4	Germination at 24, 48 and 72 h	Kernel N	**0.75**, 0.63** and 0.63****
1	Germination at 24, 48 and 72 h	Absorption	**0.61*, 0.68** and 0.71****
2	Germination at 24, 48 and 72 h	Absorption	**0.64**, 0.67** and 0.66****
3	Germination at 24, 48 and 72 h	Absorption	**0.59*, 0.61* and 0.67****
3	Germination at 24, 48 and 72 h	Leaf N (treatment plot four)	**0.61*, 0.60* and 0.68****

**** p≤0.01, * p≤0.05**

4.3.7.3 Absorption

A significant relationship (P ≤ 0.01) existed between kernel N and absorption at treatment two (Table 4.17). A significant relationship (P ≤ 0.01) existed between leaf N and absorption at treatment two, treatment plot three (N applied at planting only).

Table 4.17. Significant correlations between absorption and other malting quality traits for the two-row population at different N treatments over localities

Treatment	Malting quality trait 1	Malting quality trait 2	Correlation value
2	Absorption	Kernel N	**0.86****
2	Absorption	Leaf N (treatment plot three)	**0.54***

**** p≤0.01, * p≤0.05**

4.3.8 Linear correlations for six-row population over years

4.3.8.1 Kernel plumpness

A significant relationship (P ≤ 0.01) existed between kernel plumpness and yield at all treatments (Table 4.18). There was a significant relationship between kernel plumpness and germination at 24, 48 and 72 h at all treatments. There was a significant relationship (P ≤ 0.01) between kernel plumpness and absorption at treatment one. There was a significant correlation (P ≤ 0.01) between kernel plumpness and kernel N at treatment one. There was a significant negative correlation (P ≤ 0.05) between leaf N and kernel plumpness at treatment three, treatment plot three (half the N applied at planting and half at flag leaf stage).

Table 4.18. Significant correlations between kernel plumpness and other malting quality traits for the six-row population at different N treatments over years

Treatment	Malting quality trait 1	Malting quality trait 2	Correlation value
1	Kernel plumpness	Yield	**0.58****
2	Kernel plumpness	Yield	**0.53****
3	Kernel plumpness	Yield	**0.45****
4	Kernel plumpness	Yield	**0.41****
1	Kernel plumpness	Germination at 24, 48 and 72 h	**0.31**, 0.34** and 0.37****
2	Kernel plumpness	Germination at 24, 48 and 72 h	**0.17*, 0.22* and 0.24***
3	Kernel plumpness	Germination at 24, 48 and 72 h	**0.24**, 0.25** and 0.29****
4	Kernel plumpness	Germination at 24, 48 and 72 h	**0.43**, 0.42** and 0.42****
1	Kernel plumpness	Absorption	**0.34****
1	Kernel plumpness	Kernel N	**0.27****
3	Kernel plumpness	Leaf N (treatment plot three)	**-0.20***

**** p≤0.01, * p≤0.05**

4.3.8.2 Germination

There was a significant relationship between kernel N and germination at 24, 48 and 72 h treatments at treatments one and three (Table 4.19). There were significant correlations between all three germination tests at 24, 48 and 72 h for all treatments. A significant relationship existed between absorption and germination at 24, 48, and 72 h at treatments one and two. There was a significant relationship between leaf N and germination at 24, 48, and 72h at treatment one, treatment plot two (half the N applied at planting only); at treatment 2, treatment plots three and four (half the N applied at planting only) and at treatment four, treatment plot two (half the N applied at planting and half at six-leaf stage). A negative correlation existed between leaf N and germination at treatment four, treatment plot four (half the N applied at planting and half when 50% spikes were visible).

Table 4.19. Significant correlations between germination and other malting quality traits for the six-row population at different N treatments over years

Treatment	Malting quality trait 1	Malting quality trait 2	Correlation value
1	Germination at 24, 48 and 72 h	Kernel N	**0.29**, 0.31** and 0.32****
3	Germination at 24, 48 and 72 h	Kernel N	**0.18*, 0.21* and 0.21***
1	Germination at 24, 48 and 72 h	Absorption	**0.42**, 0.44** and 0.45****
2	Germination at 24, 48 and 72 h	Absorption	**0.35**, 0.34** and 0.28****
1	Germination at 24, 48 and 72 h	Leaf N (treatment plot two)	**0.22*, 0.24** and 0.27****
2	Germination at 24, 48 and 72 h	Leaf N (treatment plot three)	**0.29**, 0.29** and 0.25****
2	Germination at 24, 48 and 72 h	Leaf N (treatment plot four)	**0.18*, 0.19* and 0.22***
4	Germination at 24 and 48 h	Leaf N (treatment plot two)	**0.31* and 0.32***
4	Germination at 24, 48 and 72 h	Leaf N (treatment plot four)	**-0.31*, -0.31* and -0.34****

**** p≤0.01, * p≤0.05**

4.3.8.3 Absorption

A significant relationship (P ≤ 0.01) existed between kernel N and absorption at treatments one, two and three (Table 4.20). A significant relationship (P ≤ 0.01) existed between leaf N and absorption at treatments one, treatment plot one (all the N applied at planting), two, three and four (half the N applied at planting only) and treatment two, treatment plots three and four (half the N applied at planting only). A negative correlation existed between leaf N and absorption at treatment four, treatment plot four (half the N applied at planting and half when 50% spikes were visible).

Table 4.20. Significant correlations between absorption and other malting quality traits for the six-row population at different N treatments over years

Treatment	Malting quality trait 1	Malting quality trait 2	Correlation value
1	Absorption	Kernel N	**0.51****
2	Absorption	Kernel N	**0.24****
3	Absorption	Kernel N	**0.26****
1	Absorption	Leaf N (treatment plot one)	**0.18***
1	Absorption	Leaf N (treatment plot two)	**0.38****
1	Absorption	Leaf N (treatment plot three)	**0.19****
1	Absorption	Leaf N (treatment plot four)	**0.26****
2	Absorption	Leaf N (treatment plot three)	**0.36****
2	Absorption	Leaf N (treatment plot four)	**0.18****
4	Absorption	Leaf N (treatment plot four)	**-0.26***

**** p≤0.01, * p≤0.05**

4.3.9 Linear correlations for six-row population over localities

4.3.9.1 Kernel plumpness

A significant relationship (P ≤ 0.01) existed between kernel plumpness and yield at treatments one, two and three (Table 4.21). There was a significant relationship between kernel plumpness and germination at 24, 48 and 72 h at treatments one and two. A significant relationship (P ≤ 0.01) existed between kernel plumpness and absorption at treatments one, two and three. There was a significant correlation (P ≤ 0.05) between kernel plumpness and kernel N at treatment one, two and three. There was a significant correlation (P ≤ 0.01) between leaf N and kernel plumpness at treatment three, treatment plots two (half the N applied at planting and half at six-leaf stage) and three (half the N applied at planting and half at flag leaf stage) and at treatment plot four (half the N applied at planting only).

Table 4.21. Significant correlations between kernel plumpness and other malting quality traits for the six-row population at different N treatments over localities

Treatment	Malting quality trait 1	Malting quality trait 2	Correlation value
1	Kernel plumpness	Yield	**0.65****
2	Kernel plumpness	Yield	**0.64****
3	Kernel plumpness	Yield	**0.58****
1	Kernel plumpness	Germination at 24, 48 and 72 h	**0.39**, 0.42** and 0.47****
2	Kernel plumpness	Germination at 24, 48 and 72 h	**0.39**, 0.44** and 0.50****
1	Kernel plumpness	Absorption	**0.56****
2	Kernel plumpness	Absorption	**0.46****
3	Kernel plumpness	Absorption	**0.39****
1	Kernel plumpness	Kernel N	**0.46****
2	Kernel plumpness	Kernel N	**0.38****
3	Kernel plumpness	Kernel N	**0.26****
3	Kernel plumpness	Leaf N (treatment plot two)	**0.44****
3	Kernel plumpness	Leaf N (treatment plot three)	**0.26****
3	Kernel plumpness	Leaf N (treatment plot four)	**0.27****

**** p≤0.01, * p≤0.05**

4.3.9.2 Germination

There was a significant relationship between kernel N and germination at 24, 48 and 72 h treatments at treatments one and two (Table 4.22). There were significant correlations between all three germination tests at 24, 48 and 72 h for treatments one, two and three. A significant relationship existed between absorption and germination at 24, 48, and 72 h at treatments one and two. There was significant relationship between leaf N and germination at 24 and 48 h at treatment one, treatment plot one (all the N applied at planting).

Table 4.22. Significant correlations between germination and other malting quality traits for the six-row population at different N treatments over localities

Treatment	Malting quality trait 1	Malting quality trait 2	Correlation value
1	Germination at 24, 48 and 72 h	Kernel N	**0.45**, 0.48** and 0.51****
2	Germination at 24, 48 and 72 h	Kernel N	**0.59**, 0.60** and 0.59****
1	Germination at 24, 48 and 72 h	Absorption	**0.47**, 0.51** and 0.55****
2	Germination at 24, 48 and 72 h	Absorption	**0.73**, 0.74** and 0.74****
1	Germination at 24 and 48 h	Leaf N (treatment plot one)	**0.17* and 0.17***

**** p≤0.01, * p≤0.05**

4.3.9.3 Absorption

There was a significant relationship ($P \leq 0.01$) between kernel N and absorption at treatments one, two and three (Table 4.23). A significant relationship ($P \leq 0.01$) existed between leaf N and absorption at treatment one, treatment plot two (half the N applied at planting only) and treatment three, treatment plots three (half the N applied at planting and half at flag leaf stage) and four (half the N applied at planting only).

Table 4.23. Significant correlations between absorption and other malting quality traits for the six-row population at different N treatments over localities

Treatment	Malting quality trait 1	Malting quality trait 2	Correlation value
1	Absorption	Kernel N	**0.68****
2	Absorption	Kernel N	**0.74****
3	Absorption	Kernel N	**0.46****
1	Absorption	Leaf N (treatment plot two)	**0.22****
3	Absorption	Leaf N (treatment plot three)	**0.50****
3	Absorption	Leaf N (treatment plot four)	**0.43****

**** p≤0.01, * p≤0.05**

4.4 Discussion

The two- and six-row populations responded completely differently to N fertilizer applications at the different plant growth development stages for all malting quality parameters at the different environments. Kernel plumpness and yield were influenced by the different N treatments within each locality i.e. at Vaalharts and Rietriver in 2007. Single entries for these traits in the two- and six-row populations varied in their response to each N treatment over years and localities due to genotypes rather than environmental influence.

A split N application at planting and six-leaf stage influenced kernel plumpness of the two-row population at all environments. This stage of development also represents the end of tillering where the number of kernels/spike is increased as a result of additional N being applied and thus leads to an increase in yield (Baethgen *et al*., 1995). Variation in kernel plumpness was explained by yield for the two-row population when half the N was applied at planting and half at flag leaf stage across all environments. In this case, the application at planting may have enhanced tiller development but may not have been sufficient to enhance the development of kernels. Thus, a large number of smaller kernels were produced which decreased kernel plumpness but still enhanced yield (Baethgen *et al*., 1995).

From this study it was clear that applying all the fertilizer at planting or a split N application at planting and when 50% spikes are visible does not play a role in enhancing kernel plumpness. Applying all N at planting may enhance tiller development but many of these tillers may not produce spikes, as there may be competition between the spikes for water and nutrients. Tillers may also compete for light, resulting in taller and thinner stems which may lead to lodging (Baethgen *et al*., 1995). A split application of N fertilizer at planting and when 50% spikes are visible may increase yield but may also increase kernel N content thus compromising malting quality (Chen *et al*., 2006).

For the six-row population, kernel plumpness responded differently to the N treatments at all environments. Variation in kernel plumpness was explained by yield for treatments one, two and three. This may be due to the greater variation in grain size in six-row barley compared to two-row barley (Ellis and Marshall, 1998). High yields were obtained for the two- and six-row populations at Vaalharts in 2006 when N was applied at planting and when 50% spikes were visible. However the lowest yields occurred in the following season possibly due to unfavourable environmental conditions.

A negative correlation existed between leaf N and kernel plumpness for the two-row population at treatment two over localities. This relationship strengthens the results obtained when a correlation occurred between leaf N and kernel N at treatment two for the two-row population over years and localities as low kernel N content is associated with plump kernels (Swanston *et al*., 1995). A positive correlation occurred between leaf N and kernel plumpness for the six-row population at treatment three over years and localities. Application of N at flag leaf stage may have increased dry matter accumulation in the kernel thus resulting in increased kernel plumpness. At this stage the availability of N from the soil may explain the increase in leaf N. If N is still available during grain filling, kernel N would increase and kernel plumpness will decrease (Sardana and Zhang, 2005a; b).

A relationship existed between kernel N and kernel plumpness at treatment two for the two-row population and at treatment one for the six-row population over years and at treatments one, two and three for the six-row population over localities. A possible explanation for this relationship may be due to there being abundant amounts of N available in the soil. Hence, plants were able to extract as much N from the soil as

possible, as a result, as kernel plumpness increased, kernel N also increased however, the kernel can only take in a certain amount of N after which an increase in N fertilizer will not have an effect on kernel N content.

Kernel N and protein were influenced by the four N treatments applied over years in Vaalharts but not over localities. The N treatments influenced kernel N and protein within each year i.e. in 2006 and 2007 at Vaalharts and within each locality i.e. at Vaalharts and Rietriver in 2007. Kernel N variation for single entries occurred within each N treatment applied over years in Vaalharts mostly due to environmental influence rather than genotype influence.

A split N application at planting and six-leaf stage influenced kernel N for both populations at Rietriver in 2007. At Vaalharts in 2006 a split application at planting and flag leaf stage resulted in low kernel N content however, this did not occur the following season. The difference in response to N application over seasons may be due to variation in available N in the soil and environmental conditions (Agu and Palmer, 2001). Kernel N variation was explained by absorbance and kernel plumpness for treatment one and by absorbance for treatment three for the two-row population. Kernel N was explained by absorbance for treatments one, three and four for the six-row population. Long and narrow kernels impede water absorption during steeping and have high kernel protein content compared to short and plump kernels (Gebhardt *et al*., 1993).

Germination rate of the two-row population was influenced by a split N application at planting and six-leaf stage at Vaalharts in 2006 and 2007 and at planting and flag leaf at Rietriver in 2007. For the six-row population, a split N application at planting and flag leaf influenced the germination rate at all environments. Correlation analysis indicated a relationship between kernel plumpness and germination for the two-row population at treatments one and two and for the six-row population at all treatments over years and as well as, for the two-row population at treatments two and four and for the six-row population at treatments one and two over localities. Short and plump kernels have a lower kernel N content thereby facilitating endosperm modification during malting and increases malt extract (Gebhardt *et al*., 1993). There were no differences between the three germination tests conducted two weeks from each other. This proved that there were no dormancy effects in the period of malt quality analysis. However, it is not known

if a longer waiting period between germination tests will produce similar results. This was also observed for the six-row population over years and localities.

For absorption rate the best N treatments for two- and six-row populations were a split application between planting and flag leaf stage and between planting and six-leaf stage respectively at Vaalharts in 2007. Absorbance increases as germination rate increases at treatments one, three and four over years and at treatments one, two and three over localities for the two-row population and at treatments one and two over years and localities for the six-row population. A relationship also existed between kernel plumpness and absorption at treatment one over years and treatments one, two and three over localities. Water absorption allows for the hydrolytic enzymes to break down starch into sugars for the developing embryo (Yan *et al.*, 1999; Koning, 2006).

A relationship also existed between leaf N and germination and leaf N and absorption for both populations over years and localities for the different N treatments. In Chapter 3 correlations were determined between leaf N and kernel N based on the average N content for each population. However the single plants in the population responded differently to N application at the different plant growth stages. As a result it was impossible to sample leaves randomly from a population to manipulate kernel N content and ultimately germination and absorption rates.

The correlation and interaction between kernel N and germination and absorption will depend on a number of factors. The outer layer of the kernel must be modified as it may form a barrier limiting the movement of the hydrolytic enzymes necessary for the digestion of proteins and starch during germination. High amounts of protein molecules to starch granules may limit the access of hydrolytic enzymes thus reducing endosperm modification. A strong bond between starch granules and the protein matrix have been related to poor malting barleys. High kernel N content in barley samples have shown slower rates of endosperm modification compared to low kernel N samples (Brennan *et al.*, 1996; Agu and Palmer, 2001).

This study indicated that a practical N fertilizer strategy should include half N application at planting (55 kg/ha) for crop and tiller development and a split application of the other 55 kg/ha at six-leaf and flag leaf stage to enhance kernel plumpness, germination,

absorption and yield and maintain optimum kernel N for good malting quality. With further studies the ratio of N to be applied between the split application at the six-leaf and flag leaf stage needs to be optimised.

CHAPTER 5

Comparison between the entries (genotypes) within the two- and six-row doubled haploid populations in response to nitrogen fertilizer applications for malting quality

5.1 Introduction

Barley malting quality is controlled by many genes (quantitative traits) and is influenced by the environment (Fox *et al*., 2003). Hence, different genotypes may respond differently to various environments (Kaczmarek *et al*., 1999). Genotype x environment (G x E) interaction causes unpredictable variation in quantitative malting quality traits (Molina-Cano *et al*., 1997).

Malting quality is affected by environmental variation caused mostly by high temperatures and water deficit during grain filling (Passarella *et al*., 2005). High temperatures during grain filling result in a decrease in grain size and an increase in kernel protein content (Correll *et al*., 1994; Passarella *et al*., 2005). Small kernels have higher nitrogen content than larger kernels (Agu and Palmer, 2003). Long periods of heat stress reduces grain weight as the enzymes involved in starch synthesis may be damaged and/or the number and size of endosperm cells and starch granules may be reduced. Water stress is known to reduce the rate of photosynthesis and cause premature senescence of photosynthetic organs. Under severe water stress rapid desiccation may reduce nitrogen translocation from the leaves to the kernels (Savin and Nicolas, 1996).

The main aim of barley breeding programmes is to produce cultivars that are high yielding and stable in a wide range of environments. Doubled haploids have been applied in breeding programmes as these lines are homozygous and homogenuous (Kaczmarek *et al*., 1999). The purpose of this study was to determine whether there are any differences between lines within a doubled haploid population for malting quality and to determine the response of these lines to different timings of N applications.

5.2 Materials and methods

5.2.1 Materials

As explained in Chapter 3.

5.2.2 Methods

Analysis of variance (ANOVA) was done to determine the genetic variation within the two- and six-row doubled haploid populations for the different malting traits tested in this study as affected by the different N treatments applied across all environments. ANOVA was performed using GenStat® (Payne *et al*., 2007). The experiment was repeated over three environments and these were used as replications. ANOVA over years and localities was performed with Agrobase® (Mulitze, 2000).

5.3 Results

5.3.1 Simple ANOVA analysis across all environments

5.3.1.1 Kernel plumpness

There were significant differences between entries for both the two- and six-row populations (Table 5.1). The percentage of two-row entries for kernel plumpness was higher at N treatment two (57.14%) compared to treatments one (28.57%) and four (14.29%) (Table 5.2). However, only 57.14% of two-row entries were within the accepted specification (i.e. >80%) for malting quality. Only seven entries made up the two-row population. It is not known if a larger population will respond differently to the timing of N treatments and result in a higher number of entries with kernel plumpness within the required specification for malting quality. The percentage of six-row entries for kernel plumpness was higher at N treatment one (35.82%) compared to treatments two (29.85%), three (19.40%) and four (14.93%) (Table 5.3). However, only 17.91% of six row-entries were within the accepted specification (i.e. >70%) for malting quality.

Table 5.1. ANOVA for kernel plumpness of two- and six-row entries across environments

	Two-row population		Six-row population	
Source	Mean square	Coefficient of determination η^2 (%) †	Mean square	Coefficient of determination η^2 (%) †
Treatment	855.20^{ns}	5.18	1070.80^{ns}	0.83
Entry	**2903.10****	**35.20**	**1352.90****	**23.01**
Treatment x entry	300.90^{ns}	10.95	294.10^{ns}	15.01
LSD for entry	24.13		23.70	

**** p≤0.01**, ^{ns} not significant

† η^2 is defined as 100 x Factor sum of squares/total sum of squares (Molina-Cano *et al.*, 1997)

Table 5.2. Kernel plumpness of two-row entries as improved by the four different N treatments across environments of a total population of seven entries

Treatment	1	2	3	4
Entries with kernel plumpness **>80%**	**56 and 67**	**12**	**-**	**78**
Entries with kernel plumpness <80%	**-**	7, 34 and 68	**-**	**-**
Total no. of entries per treatment (%)	28.57	57.14	-	14.29

Note: Values in **bold** were within the accepted specification (i.e. >80%) for kernel plumpness of two-row entries

Table 5.3. Kernel plumpness of six-row entries as improved by the four different N treatments across environments of a total population of 67 entries

Treatment	1	2	3	4
Entries with kernel plumpness **>70%**		**8, 11, 31 and 43**	**-**	**59**
Entries with kernel plumpness <70%	3, 4, 5, 6, 13, 16, 18, 19, 21, 22, 27, 29, 36, 39, 45, 47, 49, 52, 60, 66, 69, 71, 75 and 77	17, 25, 26, 28, 38, 50, 54, 55, 58, 61, 62, 63, 64, 70, 72 and 73	10, 14, 15, 20, 30, 37, 40, 41, 42, 44, 51, 57 and 76	24, 32, 33, 35, 46, 48, 53, 65 and 74
Total no. of entries per treatment (%)	35.82	29.85	19.40	14.93

Note: Values in **bold** were within the accepted specification (i.e. >70%) for kernel plumpness of six-row entries

5.3.1.2 Germination

There were no significant differences for germination rate for the two-row entries at 24, 48 and 72 h for all three germination tests (Table 5.4 - 5.6). However, there were significant differences between entries for the six-row population at 72 h for germination test one (Table 5.7). Complete germination (i.e. 100%) was achieved by entries 6 at treatment four, 11 and 59 at treatment one and 71 at treatment three (i.e. 5.97% of six-row entries). For germination test two, there were significant differences between the six-row entries also at 72 h (Table 5.8). Complete germination was achieved by entries 32 and 36 at treatment four (i.e. 2.99% of six-row entries).

Table 5.4. ANOVA for germination test 1 of two-row entries across environments

	24 h		48 h		72 h	
Source	Mean square	Coefficient of determination η^2 (%) †	Mean square	Coefficient of determination η^2 (%) †	Mean square	Coefficient of determination η^2 (%) †
Treatment	506.00^{ns}	3.42	358.20^{ns}	3.07	304.00^{ns}	3.78
Entry	201.20^{ns}	2.72	317.00^{ns}	5.44	230.20^{ns}	5.73
Treatment x entry	235.60^{ns}	9.56	243.40^{ns}	12.53	257.70^{ns}	19.23

^{ns} not significant

† η^2 is defined as 100 x Factor sum of squares/total sum of squares (Molina-Cano *et al*., 1997)

Table 5.5. ANOVA for germination test 2 of two-row entries across environments

	24 h		48 h		72 h	
Source	Mean square	Coefficient of determination η^2 (%) †	Mean square	Coefficient of determination η^2 (%) †	Mean square	Coefficient of determination η^2 (%) †
Treatment	167.10^{ns}	1.00	96.30^{ns}	0.74	113.80^{ns}	1.15
Entry	783.30^{ns}	9.35	544.30^{ns}	8.39	373.90^{ns}	7.56
Treatment x entry	508.70^{ns}	18.21	406.50^{ns}	18.79	398.50^{ns}	24.17

^{ns} not significant

† η^2 is defined as 100 x Factor sum of squares/total sum of squares (Molina-Cano *et al*., 1997)

Table 5.6. ANOVA for germination test 3 of two-row entries across environments

	24 h		48 h		72 h	
Source	Mean square	Coefficient of determination η^2 (%) †	Mean square	Coefficient of determination η^2 (%) †	Mean square	Coefficient of determination η^2 (%) †
Treatment	95.50^{ns}	1.02	17.50^{ns}	0.21	17.90^{ns}	0.25
Entry	349.90^{ns}	7.46	165.90^{ns}	4.03	282.90^{ns}	8.06
Treatment x entry	440.90^{ns}	28.20	315.60^{ns}	23.02	186.40^{ns}	15.92

^{ns} not significant

† η^2 is defined as 100 x Factor sum of squares/total sum of squares (Molina-Cano *et al*., 1997)

Table 5.7. ANOVA for germination test 1 of six-row entries across environments

	24 h		48 h		72 h	
Source	Mean square	Coefficient of determination η^2 (%) †	Mean square	Coefficient of determination η^2 (%) †	Mean square	Coefficient of determination η^2 (%) †
Treatment	1063.50^{ns}	0.83	862.60^{ns}	0.76	732.70^{ns}	0.75
Entry	668.70^{ns}	11.52	670.50^{ns}	12.95	**643.20****	14.55
Treatment x entry	346.80^{ns}	17.93	314.60^{ns}	18.24	286.50^{ns}	19.44
LSD for entry	-		-		21.27	

** **p≤0.01**, ^{ns} not significant

† η^2 is defined as 100 x Factor sum of squares/total sum of squares (Molina-Cano *et al.*, 1997)

Table 5.8. ANOVA for germination test 2 of six-row entries across environments

	24 h		48 h		72 h	
Source	Mean square	Coefficient of determination η^2 (%) †	Mean square	Coefficient of determination η^2 (%) †	Mean square	Coefficient of determination η^2 (%) †
Treatment	1657.00^{ns}	1.24	927.60^{ns}	0.77	637.20^{ns}	0.58
Entry	780.90^{ns}	12.82	729.90^{ns}	13.26	**703.00****	14.10
Treatment x entry	355.90^{ns}	17.52	327.00^{ns}	17.83	319.70^{ns}	19.24
LSD for entry	-		-		22.84	

** **p≤0.01**, ^{ns} not significant

† η^2 is defined as 100 x Factor sum of squares/total sum of squares (Molina-Cano *et al.*, 1997)

For germination test three, there were significant differences between six-row entries for germination rate at 24, 48 and 72 h (Table 5.9). At 24, 48 and 72 h only entry 49 achieved 100% germination at N treatment three. Six-row entries 3, 4, 5 and 31 performed consistently for all three germination tests with germination rates ranging from 87.50 - 93.33%. Entries 18, 19, 42 and 44 performed consistently poorly for all germination tests ranging from 59.17 - 69.58%.

Table 5.9. ANOVA for germination test 3 of six-row entries across environments

	24 h		48 h		72 h	
Source	Mean square	Coefficient of determination η^2 (%) †	Mean square	Coefficient of determination η^2 (%) †	Mean square	Coefficient of determination η^2 (%) †
Treatment	593.10^{ns}	0.50	548.50^{ns}	0.52	365.30^{ns}	0.37
Entry	**737.10****	13.70	**698.30****	14.47	**641.10****	14.11
Treatment x entry	402.10^{ns}	22.43	334.80^{ns}	20.81	321.50^{ns}	21.22
LSD for entry	23.13		22.07		21.42	

**** p≤0.01**, ^{ns} not significant

† η^2 is defined as 100 x Factor sum of squares/total sum of squares (Molina-Cano *et al.*, 1997)

5.3.1.3 Other malting quality traits

Nitrogen fertilizer treatments did not have a significant influence on the two- and six-row entries for yield, absorption and kernel N across all environments tested.

5.3.2 Simple ANOVA analysis over years

5.3.2.1 Kernel plumpness

There were significant differences between two- and six-row entries (Table 5.10). Genotypes contributed 80.1% of the total variation among the entries compared to the environment (19.9%). The percentage of two-row entries for kernel plumpness was higher at N treatment two (42.86%) compared to treatments one (28.57%), three (14.29%) and four (14.29%) (Table 5.11). However, only 57.14% of two-row entries were within the accepted specification (i.e. >80%) for malting quality. Two-row entries 12 and 78 also differed with entries 34 and 68. The percentage of six-row entries for kernel plumpness was higher at N treatment four (29.85%) compared to treatments one (28.36%), two (25.37%) and three (16.42%) (Table 5.12). However, only 8.96% of six-row entries were within the accepted specification (i.e. >70%) for malting quality.

Table 5.10 ANOVA for kernel plumpness of two- and six-row entries over years

Source	Mean square	Coefficient of determination η^2 (%) †
Treatment	67.43^{ns}	0.07
Year in treatment	334.98^{ns}	0.49
Entry	**1179.01****	**33.08**
Treatment x entry	234.94^{ns}	19.77
LSD for entry	16.81	
Heritability	0.80	

**** p≤0.01**, ^{ns} not significant

† η^2 is defined as 100 x Factor sum of squares/total sum of squares (Molina-Cano *et al*., 1997)

Table 5.11. Kernel plumpness of two-row entries as affected by the four different N treatments over years of a total population of seven entries

Treatment	1	2	3	4
Entries with kernel plumpness **>80%**	-	**7, 12 and 78**	**56**	-
Entries with kernel plumpness <80%	67 and 68	-	-	34
Total no. of entries per treatment (%)	28.57	42.86	14.29	14.29

Note: Values in **bold** were within the accepted specification (i.e. >80%) for kernel plumpness of two-row entries

Table 5.12. Kernel plumpness of six-row entries as affected by the four different N treatments over years of a total population of 67 entries

Treatment	1	2	3	4
Entries with kernel plumpness **>70%**	**60**	**8 and 21**	**-**	**11, 59 and 77**
Entries with kernel plumpness <70%	4, 10, 16, 18, 26, 27, 28, 29, 38, 39, 42, 45, 49, 51, 66, 69, 70 and 71	15, 17, 19, 25, 31, 40, 43, 50, 54, 55, 61, 62, 64, 72 and 73	5, 14, 24, 30, 37, 41, 44, 52, 63, 75 and 76	3, 6, 13, 20, 22, 32, 33, 35, 36, 46, 47, 48, 53, 57, 58, 65 and 74
Total no. of entries per treatment (%)	28.36	25.37	16.42	29.85

Note: Values in **bold** were within the accepted specification (i.e. >70%) for kernel plumpness of six-row entries

5.3.2.2 Yield

There were significant differences between entries (Table 5.13). Genotypes contributed 61.9% of the total variation among the entries whilst 38.1% was due to environmental influence. The percentage of two-row entries for yield was equally improved by N treatments two, three and four (28.57%) compared to treatment one (14.30%) (Table 5.14). However, only 28.57% of two-row entries were within the accepted specification (i.e. >8.00 t/ha) for malting quality. Two-row entries 12, 56 and 78 differed from entry 68 and entry 12 also differed from entry 34. The percentage of six-row entries for yield was higher at N treatment two (31.34%) compared to treatments three (26.87%), four (23.88%) and one (17.91%) (Table 5.15). However, only 31.34% of six-row entries were within the required specification (i.e. >8.00 t/ha) for malting quality.

Table 5.13 ANOVA for yield of two- and six-row entries over years

Source	Mean square	Coefficient of determination η^2 (%) †
Treatment	14.49^{ns}	0.71
Year in treatment	17.42^{ns}	1.14
Entry	**19.38****	24.20
Treatment x entry	7.39^{ns}	27.67
LSD	2.51	
Heritability	0.62	

**** p≤0.01**, ^{ns} not significant

† η^2 is defined as 100 x Factor sum of squares/total sum of squares (Molina-Cano *et al*., 1997)

Table 5.14. Yield of two-row entries as affected by the four different N treatments over years of a total population of seven entries

Treatment	1	2	3	4
Entries with yield **>8 t/ha**	**-**	**12**	**56**	**-**
Entries with yield <8 t/ha	7	78	68	34 and 67
Total no. of entries per treatment (%)	14.30	28.57	28.57	28.57

Note: Values in **bold** were within the accepted specification for yield (i.e. >8 t/ha)

Table 5.15. Yield of six-row entries as affected by the four different N treatments over years of a total population of 67 entries

Treatment	1	2	3	4
Entries with yield **>8 t/ha**	**10, 18, 29 and 70**	**14, 21, 32 and 43**	**5, 8, 20, 26, 30, 37, 52, 63 and 73**	**11, 22, 33 and 46**
Entries with yield <8 t/ha	27, 36, 44, 45, 48, 49, 53 and 72	15, 19, 25, 28, 31, 38, 41, 42, 54, 55, 57, 58, 62, 64, 65, 69 and 75	4, 6, 13, 24, 40, 59, 66, 71and 76	3, 16, 17, 35, 39, 47, 50, 51, 60, 61, 74 and 77
Total no. of entries per treatment (%)	17.91	31.34	26.87	23.88

Note: Values in **bold** were within the accepted specification for yield (i.e. >8 t/ha)

5.3.2.3 Kernel nitrogen

There were significant differences between entries (Table 5.16). Genotypes contributed 40.3% of the total variation among the entries. Therefore, there was 59.7% environmental influence. Kernel nitrogen for two-row entry 78 at treatment three and for the six-row entry 10 at treatment four were the only entries that were within the accepted specification for malting quality i.e. between 1.5 - 2.0%.

Table 5.16 ANOVA for kernel N of two- and six-row entries over years

Source	Mean square	Coefficient of determination η^2 (%) †
Treatment	1.06**	3.13
Year in treatment	2.60**	10.21
Entry	**0.20***	15.10
Treatment x entry	0.12^{ns}	27.02
LSD	0.32	
Heritability	0.40	

**** p≤0.01**, * p≤0.05, ^{ns} not significant

† η^2 is defined as 100 x Factor sum of squares/total sum of squares (Molina-Cano *et al.*, 1997)

5.3.3 Simple ANOVA over localities

5.3.3.1 Kernel plumpness

There were significant differences between two- and six-row entries (Table 5.17). Genotypes contributed 73.9% of the total variation among the entries whilst 26.1% was due to environmental influence. The percentage of two-row entries for kernel plumpness was equally improved by N treatments one and two (42.86%) compared to treatment four (14.29%) (Table 5.18). However, only 28.57% of two-row entries were within the accepted specification (i.e. >80%) for malting quality. In addition, entries 12 and 78 differed from entries 7, 34 and 56 and entry 78 differed from entry 67. For kernel plumpness, two-row entries 12 and 78 performed consistently over years and localities ranging from 82.70 - 94.45%, whilst entry 68 performed poorly ranging from 38.79 - 46.44%. The percentage of six-row entries for kernel plumpness was higher at N treatment one (37.31%) compared to treatments two (26.87%), three (22.39%) and four (13.43%) (Table 5.19). However, kernel plumpness was not within the accepted specification (i.e. >70%) for malting quality for all six-row entries over localities.

Table 5.17 ANOVA for kernel plumpness of two- and six-row entries over localities

Source	Mean square	Coefficient of determination η^2 (%) †
Treatment	2544.28**	1.99
Location in treatment	12589.28**	13.11
Entry	**1269.03****	**24.97**
Treatment x entry	330.68^{ns}	19.62
LSD	18.59	
Heritability	0.74	

**** p≤0.01**, ^{ns} not significant

† η^2 is defined as 100 x Factor sum of squares/total sum of squares (Molina-Cano *et al.*, 1997)

Table 5.18. Kernel plumpness of two-row entries as affected by the four different N treatments over localities of a total population of seven entries

Treatment	1	2	3	4
Entries with kernel plumpness **>80%**	**78**	**12**	**-**	**-**
Entries with kernel plumpness <80%	56 and 67	34 and 68	-	7
Total no. of entries per treatment (%)	42.86	42.86	-	14.29

Note: Values in **bold** were within the accepted specification (i.e. >80%) for kernel plumpness of two-row entries

Table 5.19. Kernel plumpness of six-row entries as affected by the four different N treatments over localities of a total population of 67 entries

Treatment	1	2	3	4
Entries with kernel plumpness **>70%**	**-**	**-**	**-**	**-**
Entries with kernel plumpness <70%	3, 5, 13, 16, 17, 18, 19, 21, 22, 29, 36, 40, 42, 44, 45, 47, 49, 53, 60, 66, 69, 70, 75, 76 and 77	8, 11, 25, 26, 27, 31, 38, 43, 50, 52, 54, 55, 58, 61, 62, 63, 72 and 73	4, 6, 10, 14, 15, 20, 28, 30, 35, 37, 39, 41, 51, 57 and 71	24, 32, 33, 46, 48, 59, 64, 65 and 74
Total no. of entries per treatment (%)	37.31	26.87	22.39	13.43

Note: Values in **bold** were within the accepted specification (i.e. >70%) for kernel plumpness of six-row entries

5.3.3.2 Yield

There were significant differences between two- and six-row entries (Table 5.20). Genotypes contributed 59.2% of the total variation among the entries compared to 40.8% of environmental influence. The percentage of two-row entries for yield was higher at N treatment two (29.85%) compared to treatments three (26.87), four (23.88%) and one (17.91%) (Table 5.21). However, yield was only within the accepted specification (i.e. >8.00 t/ha) for malting quality for two-row entry 12 (influenced by treatment two). Two-row entries 7, 12 and 56 differed from entry 68 and entries 7 and 12 also differed from entry 78.

The percentage of six-row entries for yield was higher at N treatment two (31.34%) compared to treatments one (25.37%), four (22.39%) and three (20.90%) (Table 5.22). However, only 11.94% of six-row entries were within the required specification (i.e. >8.00 t/ha) for malting quality. For yield, two-row entries 34 and 68 (ranging from 1.95 - 5.25 t/ha) and six-row entries 35, 57, 76 and 77 (ranging from 0.39 - 2.93 t/ha) performed poorly for yield over years and localities.

Table 5.20 ANOVA for yield of two- and six-row entries over localities

Source	Mean square	Coefficient of determination η^2 (%) †
Treatment	8.39^{ns}	0.34
Location in treatment	**676.05****	**36.86**
Entry	**13.97****	14.48
Treatment x entry	5.70^{ns}	17.70
LSD	2.24	
Heritability	0.59	

**** p≤0.01**, ^{ns} not significant

† η^2 is defined as 100 x Factor sum of squares/total sum of squares (Molina-Cano *et al.*, 1997)

Table 5.21. Yield of two-row entries as affected by the four different N treatments over localities of a total population of seven entries

Treatment	1	2	3	4
Entries with yield **>8 t/ha**	**-**	**12**	**-**	**-**
Entries with yield <8 t/ha	67 and 78	34 and 68	7 and 56	-
Total no. of entries per treatment (%)	17.91	29.85	26.87	23.88

Note: Values in **bold** were within the accepted specification for yield (i.e. >8 t/ha)

Table 5.22. Yield of six-row entries as affected by the four different N treatments over localities of a total population of 67 entries

Treatment	1	2	3	4
Entries with yield **>8 t/ha**	**10, 29 and 44**	**26, 32 and 52**	**30 and 73**	**-**
Entries with yield <8 t/ha	6, 16, 18, 22, 27, 36, 38, 45, 49, 51, 60, 66, 70 and 76	14, 21, 25, 28, 31, 41, 42, 43, 54, 55, 57, 58, 61, 62, 64, 65, 69 and 72	4, 5, 13, 15, 20, 35, 37, 40, 59, 63, 71 and 77	3, 8, 11,17, 19, 24, 33, 39, 46, 47, 48, 50, 53, 74 and 75
Total no. of entries per treatment (%)	25.37	31.34	20.90	22.39

Note: Values in **bold** were within the accepted specification for yield (i.e. >8 t/ha)

5.3.3.3 Kernel nitrogen

There were no significant differences in kernel N between entries over localities in 2007 (Table 5.23).

Table 5.23. ANOVA for kernel N of two- and six-row entries over localities

Source	Mean square	Coefficient of determination η^2 (%) †
Treatment	0.12^{ns}	0.15
Location in treatment	**19.31****	**30.76**
Entry	0.31^{ns}	9.23
Treatment x entry	0.26^{ns}	23.60
LSD	0.45	
Heritability	0.15	

**** p≤0.01**, ^{ns} not significant

† η^2 is defined as 100 x Factor sum of squares/total sum of squares (Molina-Cano *et al*., 1997)

5.4 Discussion

Solimon and Allard (1991) stated that genetic diversity of a population in the form of heterozygous individuals or in the form of a set of different homozygous genotypes secures their greater stability in various environmental conditions. However, in this study the two- and six-row entries in the doubled haploid population differed in malting quality across environments. All individual entries are homozygous in a doubled haploid population but differ genetically from each other, although derived from the same crossing combination due to recombination or crossing over of genes during meiotic division. The two- and six-row entries were also influenced by different N applications across environments. The variation in the doubled haploid entries may be due to the influence of the environment on the translocation ability of these entries to transport water and nutrients. Moreno *et al*. (2003) stated that the response of cereals to N depends on seasonal variations.

In this study, genotypes contributed more to variation in a population than the environment for both kernel plumpness and yield. A cross between a two-row cultivar and a six-row cultivar may result in positive transgressive segregants, however such a cross could lead to an imbalance in malting quality traits (Marquez-Cedillo *et al*., 2000;

Hayes *et al*., 2003). This explains the difference in genetic response of entries within a population. In some cases it has been reported that environmental conditions may affect kernel weight e.g. long and even short periods of high temperature stress causes acceleration in leaf senescence. The grain filling period is reduced resulting in a decrease in kernel weight and starch synthesis is also limited, however the rate of kernel development is not affected (Savin and Nicolas, 1996; Moreno *et al*., 2003).

Germination differed greatly in six-row entries compared to two-row entries. The central kernel of six-row barley is symmetrical compared to the two lateral kernels which are shorter and thinner. In contrast two-row barley kernels tend to be symmetrical, uniform and plumper because only one rachis node develops (Schwarz and Horsley, 2009). Hence the irregularities in kernel size of six-row barley may explain the variation in germination found in six-row entries compared to the two-row entries. However, the response of some six-row entries was consistent across environments. The genetic make-up of these entries may be more stable in a range of environmental conditions. Variation in germination of progeny is common especially in wild species (Ellis and Marshall, 1998).

Differences were observed for kernel N between entries in the same environment over seasons compared to different environments and hence, the environment contributed more to variation than the genotypes. Due to the polygenic nature of N translocation efficiency in barley, the environment largely contributed to the variance in kernel N for the different entries. The influence of the environment on a complex trait such as kernel N can be due to direct influence of the environment on gene expression or the direct influence of the environment on plant growth and development e.g. high temperatures during grain filling may reduce starch synthesis (Mather *et al*., 1997; Fox *et al*., 2003; Passarella *et al*., 2005). Sardana and Zhang (2005b) have reported cultivar differences for kernel protein content.

In this study, the cross made between the two-row barley cultivar, Extract which has high malt extract yield and good malt quality with the six-row barley cultivar, Excel which has good disease resistance and high yield resulted in traits from the two-row parent being transferred to the six-row progeny and vice versa. Although malt extract yield was not determined in this study, the indicators of malt extract yield were determined viz. kernel

plumpness, germination, absorption and kernel N. These parameters indicated that the two-row lines, 12 and 56 and the six-row lines, 8 and 11 possessed high yield potential and good malt quality traits for high malt extract yield and hence these six-row progeny may be used for malting. The two- and six-row lines identified may be used as parents for future crosses for developing doubled haploid populations.

Barley possesses great genetic diversity that enables it to grow in a wide range of environments and to tolerate stress such as drought and salinity. It is difficult to quantify the variation in agronomic and malting quality traits due to complex inheritance of these traits. However, the analysis of QTLs controlling different malting quality traits may explain the genetic diversity present in barley (Hayes *et al*., 2003).

CHAPTER 6

Relationship between malting quality traits and hordeins as affected by timing of nitrogen fertilizer application

6.1 Introduction

The application of nitrogen (N) fertilizer to malting barley is essential to obtain high yields but in amounts that do not affect malting quality (Thompson *et al*., 2004). Malt quality is influenced by kernel protein content (Šimić *et al*., 2007) and kernel protein content is affected by the rate and timing of N fertilizer application (Riley *et al*., 1998). High protein content reduces water uptake during germination and lowers malt extract levels. In the brewery, high protein content in kernels have a longer filtration time, beer develops cloudiness and has a shorter shelf life. Insufficient levels of protein limit yeast growth during fermentation and causes beer foam to cling to the side of the glass (Emebiri *et al*., 2005).

The negative correlation between kernel protein content and malt extract is mainly due to hordeins which are the major fraction of endosperm storage proteins (Bulman *et al*., 1994). Hordeins are negatively correlated with starch which is the main source of malt extract. Hordeins are the major component of the endosperm protein matrix into which the starch granules are embedded, thus restricting access of amylolytic enzymes to the starch granules during germination (Šimić *et al*., 2007).

There are four types of hordeins, main types are B hordein (sulphur rich) and C hordein (sulphur poor) and minor types are γ (sulphur rich) and D hordein (high molecular weight) (Howard *et al*., 1996). An increase in N uptake increases the concentration of D hordeins and decreases malting quality. D hordeins occur below the sub-aleurone layer and are major components of the gel protein fraction. B and D hordeins form the gel protein matrix that may limit modification of barley during malting since they are held together by multiple interchain di-sulphide bonds (Molina-Cano *et al*., 2001; Celus *et al*., 2006). A low D:B hordein ratio increases malting quality while a high B:C hordein ratio increases malting quality. C hordeins increase when high levels of fertilizer are available (Savin *et al*., 2006). Similarly in wheat the high molecular weight glutenin subunits form

the protein matrix that surrounds the starch granules and determines the elasticity of dough for bread and other food. The gluten proteins therefore determine bread making quality in wheat (Shewry and Halford, 2002).

The relationship between barley protein content and malt quality has been extensively studied and previous studies on the relationship between hordein fractions and malt quality have been inconsistent (Wang *et al*., 2007). The objectives of this study were to determine the effect of N application timing on hordein fractions and to determine the relationship between hordein fractions and malt quality using reverse phase-high performance liquid chromatography (RP-HPLC).

6.2 Materials and methods

6.2.1 Materials

The two- and six-row parents (Extract and Excel respectively) and a representative for each doubled haploid progeny were used for RP-HPLC analysis. For the two-row progeny, entry 68; six-row progeny, entry 11 and mixed progeny (that is, progeny which consisted of both two- and six-row spikes on one plant), entries 9 and 23 were selected based on their excellent malting quality traits for all treatments over localities and years. Three replicates were analyzed per entry at three environments viz. Vaalharts in 2006, Vaalharts 2007 and Rietriver 2007. Fertilizer rate and treatments one to four were as explained in Chapter 3 and 4.

6.2.2 Methods

6.2.2.1 RP-HPLC analysis

A single kernel per entry for each N treatment was used for RP-HPLC analysis. Proteins were extracted twice from a single kernel with 1 ml of 5% (w/v) NaCl at room temperature. Samples were vortexed for 2 min and stirred for 10 min. Hordeins were extracted with 500 µl of 55% (v/v) 1-propanol/1% (w/v) dithiothreitol (DTT). The suspensions were centrifuged at 1500 g for 10 min to obtain the supernatant hordeins. This extraction procedure was repeated three times at 60°C and the combined supernatants were diluted to 2 ml with distilled water (Celus *et al*., 2006).

The Thermo Finnigan™ Surveyor Plus HPLC was used for RP-HPLC analysis. Aliquots of 500 µl was filtered through 0.45 µm membranes and 50 µl injected into a YMC-Pack ODS-A C18 column. The elution solvents used were deionised water containing 0.1% (v/v) trifluroacetic acid (TFA) as solvent 1 and acetonitrile (ACN) containing 0.1% TFA (v/v) as solvent 2. Proteins were eluted with a linear gradient from 24% to 56% solvent 2 in 50 min. Proteins were detected using UV absorbance at 214 nm. The column was washed for 10 min with 90% solvent 2 and equilibrated with 24% solvent 2 after each sample was analysed. The RP-HPLC was used for both qualitative and quantitative analysis of hordeins. The hordeins were separated into three fractions, D, C and B + γ hordeins. The levels of hordein fractions were quantified by calculating the peak area of each fraction with the Chromquest™ 4.2 software and expressed in arbitrary units (Celus *et al*., 2006; Šimić *et al*., 2007). D:B and B:C hordein ratios were calculated from the areas underneath the peaks for the representative hordein groups.

6.2.2.2 Statistical analysis

Analysis of variance (ANOVA) was carried out to determine the effect of N fertilizer treatments on the different hordein fractions at three environments. ANOVA was performed using Agrobase Generation II® (Mulitze, 2008) and the factorial design was used. The interactions between malting quality traits and hordein fractions as affected by timing of N fertilizer application were determined by using simple linear correlations with the Number Cruncher Statistical System (NCSS) (Hintze, 2004).

6.3 Results

6.3.1 Influence of timing of nitrogen application on hordein fractions

6.3.1.1 Quality and quantity of hordein fractions using RP-HPLC

The hordeins were successfully separated and quantified into D, C and B + γ fractions. Hordein chromatographs obtained by Šimić *et al*. (2007) were used as a guideline to distinguish between the different hordein fractions obtained in this study. RP-HPLC chromatographs showed distinct hordein patterns for the different genotypes tested (Figures 6.1 - 6.6). For example, for C hordeins the peaks between 26 - 32 min on all chromatographs were different for each genotype (refer to arrows on each chromatograph). Although one entry represented each genotype in this study, it is shown in Figures 6.5 and 6.6 that the two entries 9 and 23, representing the mixed progeny

also differed in hordein composition. There were no changes in hordein composition in response to timing of N application and to the environment over years and localities, as a result, only one hordein chromatograph is represented for each genotype across treatments and over years and localities.

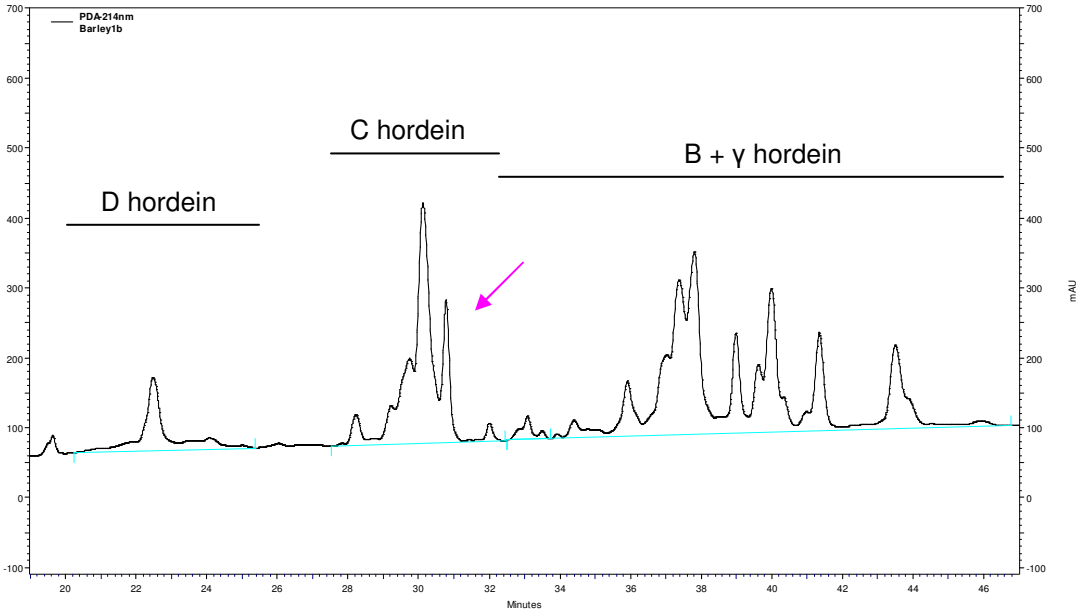

Figure 6.1. RP-HPLC chromatogram showing hordein fractions of the two-row parent

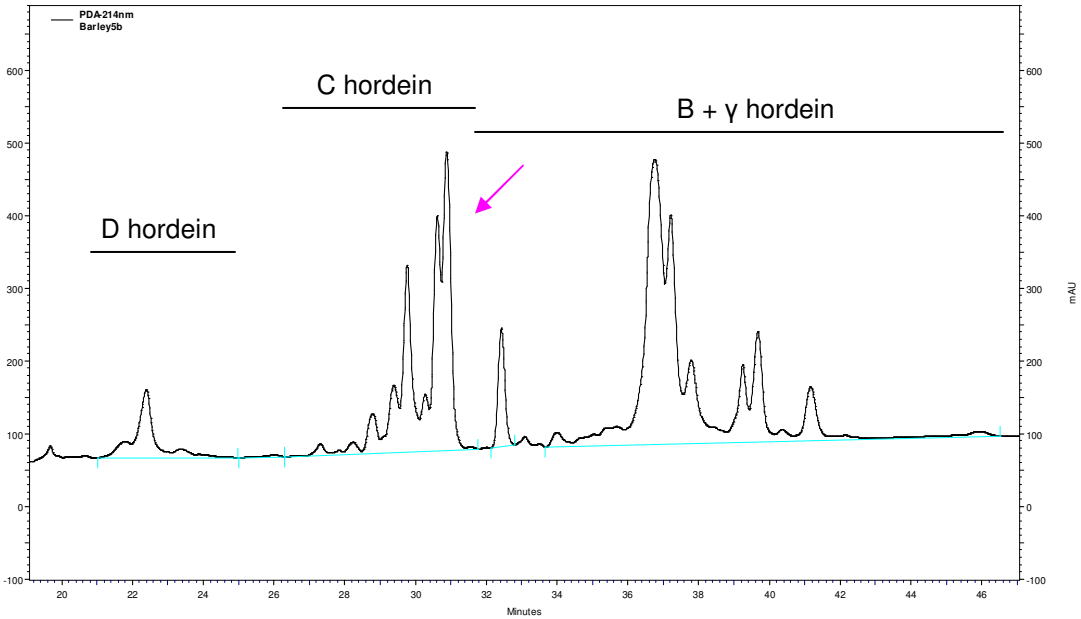

Figure 6.2. RP-HPLC chromatogram showing hordein fractions of two-row progeny

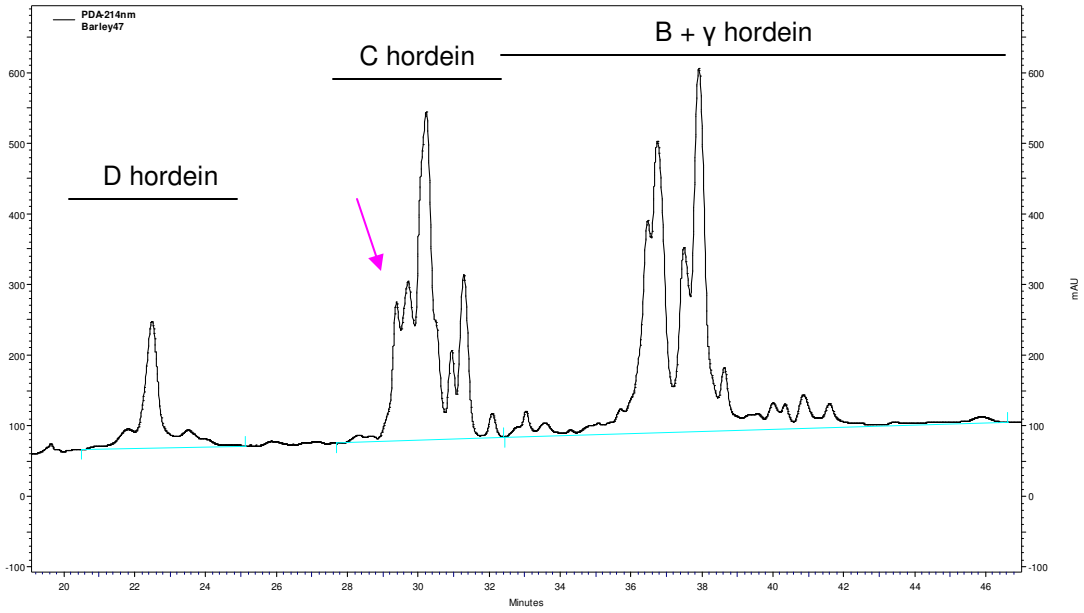

Figure 6.3. RP-HPLC chromatogram showing hordein fractions of the six-row parent

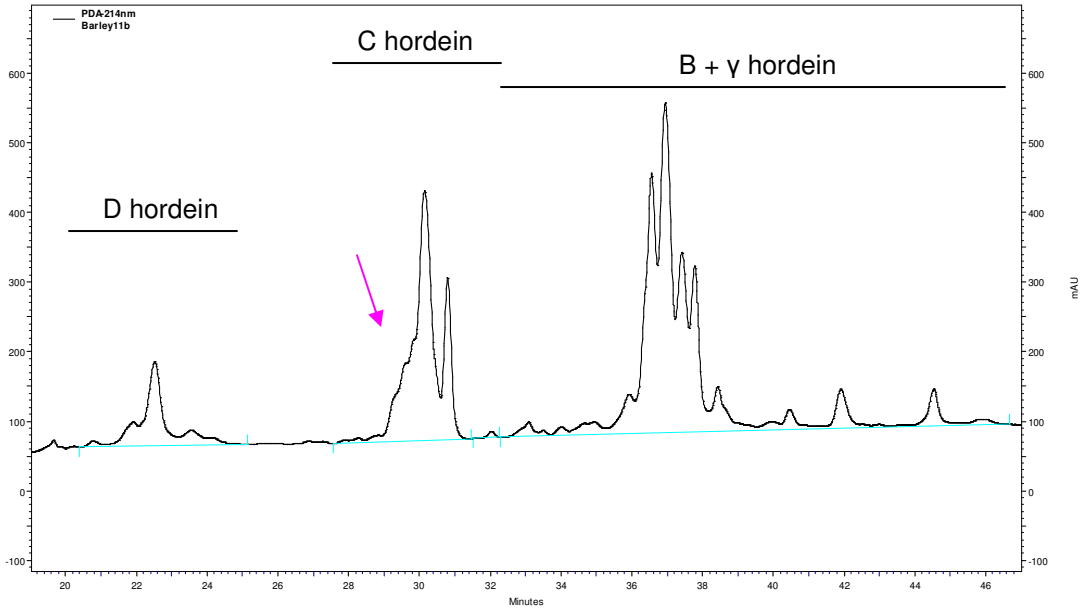

Figure 6.4. RP-HPLC chromatogram showing hordein fractions of six-row progeny

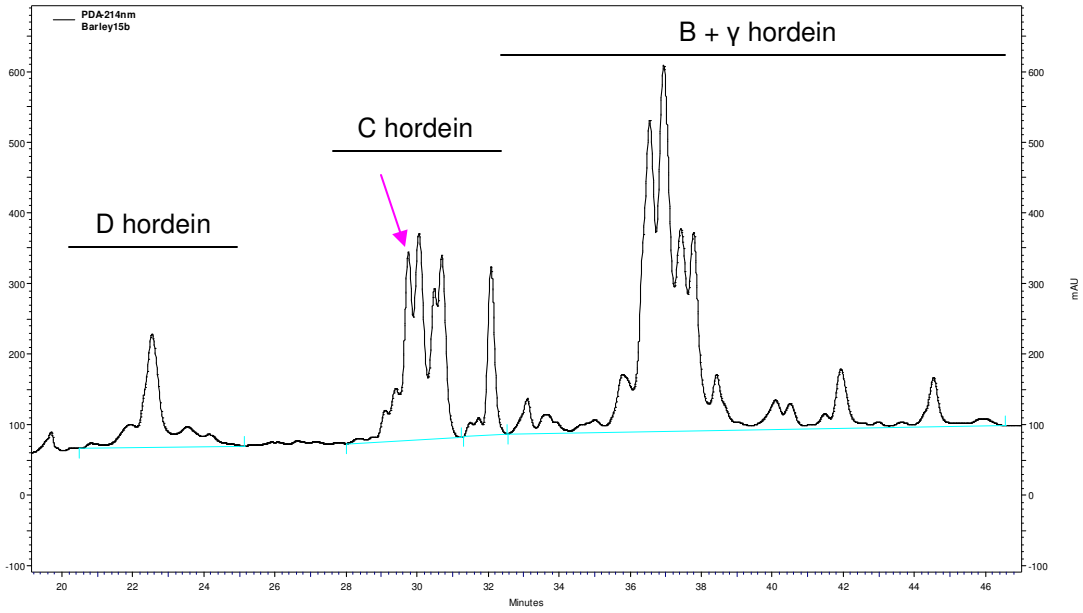

Figure 6.5. RP-HPLC chromatogram showing hordein fractions of mixed progeny (entry 9)

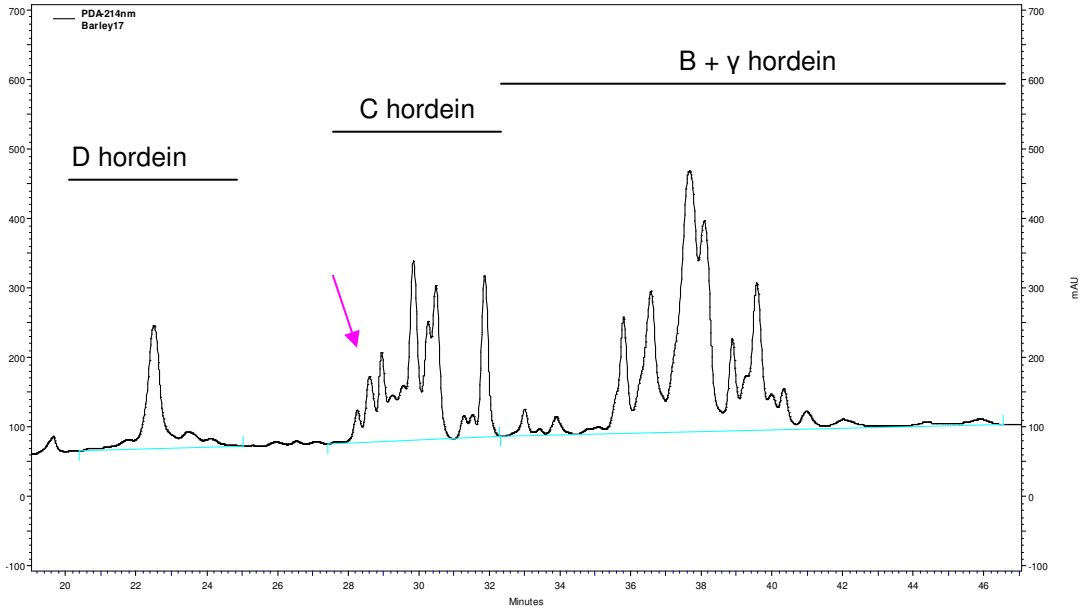

Figure 6.6. RP-HPLC chromatogram showing hordein fractions of mixed progeny (entry 23)

Averaged across treatments and environments, the two-row parent had the lowest hordein composition and the six-row progeny had the highest hordein composition as compared to the other genotypes (Figure 6.7). Total hordein composition of the two-row progeny (3.72%) and six-row parent (3.70%) were similar. However, the C hordein fraction was higher for the two-row progeny (1.34%) compared to the six-row parent (1.13%). Furthermore the B hordein fraction was higher for the six-row parent (2.28%) compared to the two-row progeny (2.08%). Total hordein composition of the mixed progeny (3.67%) was more comparable with the six-row parent (3.70%) and two-row progeny (3.72%) respectively. However, the different hordein fractions of the mixed progeny were more similar to the two-row progeny than the six-row parent.

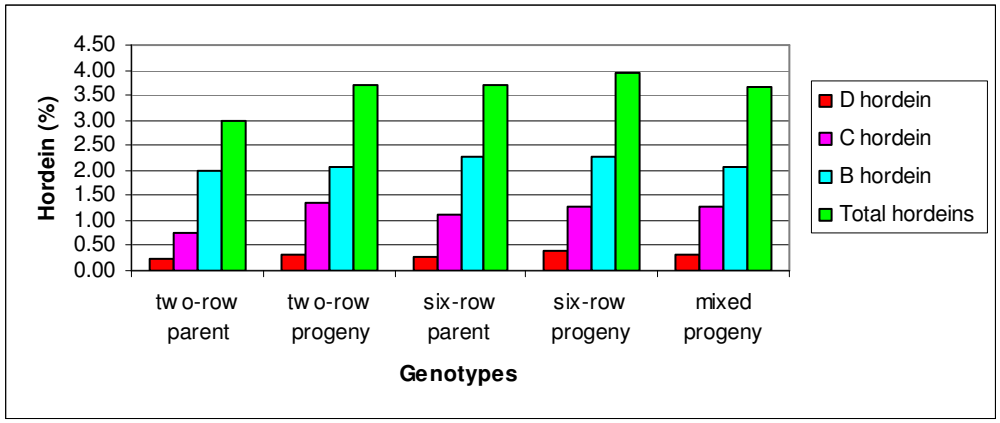

Figure 6.7. Hordein fractions for barley genotypes

6.3.1.2 Factorial ANOVA analysis

All entries differed significantly for all hordein fractions and for D:B and B:C hordein ratios at all environments except B hordeins at Vaalharts in 2006 (Table 6.1). There were significant effects of entry x treatment only for D hordeins and D:B hordein ratio at Vaalharts in 2007 and for B hordeins at Rietriver in 2007. Timing of N application only had a significant effect on C hordeins at Vaalharts in 2007.

Table 6.1. Mean square values from ANOVA for hordein fractions at different localities

Location	Vaalharts 2006				
Source	D hordeins	C hordeins	B hordeins	D:B hordein ratio	B:C hordein ratio
Entry	**589.98****	**12963.80****	1999.53^{ns}	**0.01****	**3.80****
Treatment	16.94^{ns}	219.27^{ns}	191.79^{ns}	0.00^{ns}	0.06^{ns}
Entry x treatment	67.74^{ns}	1547.62^{ns}	1602.25^{ns}	0.00^{ns}	0.12^{ns}
Location	Vaalharts 2007				
Entry	**177.58***	**11504.07****	**8381.73****	**0.01****	**1.84****
Treatment	95.26^{ns}	**5174.37***	9972.75^{ns}	0.00^{ns}	0.20^{ns}
Entry x treatment	**132.89***	1552.63^{ns}	2574.10^{ns}	**0.00****	0.22^{ns}
Location	Rietriver 2007				
Entry	**685.27****	**8830.25****	**15422.10****	**0.01****	**1.64****
Treatment	77.00^{ns}	1653.23^{ns}	1323.46^{ns}	0.00^{ns}	0.25^{ns}
Entry x treatment	44.73^{ns}	1058.97^{ns}	**1250.96***	0.00^{ns}	0.19^{ns}

**** p≤0.01, * p≤0.05**, ^{ns} not significant

6.3.1.3 Linear correlations

A significant relationship existed between C and D hordeins at all N treatments (Table 6.2). B hordeins was significantly correlated ($P \leq 0.01$) with D and C hordeins at treatments one, two and three. A significant relationship ($P \leq 0.01$) existed between total hordein content and D, B and C hordeins at all N treatments. D:B hordein ratio was significantly correlated with D hordeins at all N treatments ($P \leq 0.01$) and with total hordein content at treatment one ($P \leq 0.05$). B:C hordein ratio showed significant negative correlations with total hordein content at treatments one ($P \leq 0.05$), two and three ($P \leq 0.01$); D hordeins at treatments two and three ($P \leq 0.01$); C hordeins at all N treatments ($P \leq 0.01$) and D:B ratio at treatment four ($P \leq 0.05$).

Table 6.2. Significant correlations between hordein fractions at the different N treatments

Hordein trait 1	Hordein trait 2	Treatment	Correlation value
C hordein	D hordein	1, 2, 3 and 4	**0.70**, 0.77**, 0.64** and 0.57***
B hordein	D hordein	1, 2 and 3	**0.74**, 0.71** and 0.69****
B hordein	C hordein	1, 2 and 3	**0.68**, 0.77** and 0.76****
Total hordein	D hordein	1, 2, 3 and 4	**0.83**, 0.82**, 0.76** and 0.68****
Total hordein	C hordein	1, 2, 3 and 4	**0.93**, 0.94**, 0.94** and 0.68****
Total hordein	B hordein	1, 2, 3 and 4	**0.90**, 0.94**, 0.93** and 0.83****
D:B hordein ratio	D hordein	1, 2, 3 and 4	**0.87**, 0.69**, 0.80** and 0.77****
D:B hordein ratio	Total hordein	1	**0.52***
B:C hordein ratio	Total hordein	1, 2 and 3	**-0.59*, -0.66** and -0.73****
B:C hordein ratio	D hordein	2 and 3	**-0.62** and -0.55****
B:C hordein ratio	C hordein	1, 2, 3 and 4	**-0.80**, -0.82**, -0.88** and -0.77****
B:C hordein ratio	D:B hordein ratio	4	**-0.54***

**** p≤0.01, * p≤0.05**

6.3.2 Relationship between malting quality traits and hordein fractions

6.3.2.1 Nitrogen treatments

Kernel plumpness showed significant negative correlations with D hordeins ($P \leq 0.05$), C, B and total hordein content ($P \leq 0.01$) at treatment two (Table 6.3). Absorption showed significant positive correlation with C hordeins ($P \leq 0.05$) and negative correlation with the B:C ratio at treatment two. A significant relationship existed between leaf N at treatment plot two and C ($P \leq 0.01$) and B hordeins ($P \leq 0.05$) and total hordein content ($P \leq 0.01$) at treatment three.

Table 6.3. Significant correlations between malting quality traits and hordein fractions at the different N treatments

Malting quality trait	Hordein trait	Treatment	Correlation value
Kernel plumpness	D hordein	2	**-0.56***
Kernel plumpness	C hordein	2	**-0.74****
Kernel plumpness	B hordein	2	**-0.72****
Kernel plumpness	Total hordein	2	**-0.77****
Absorption	C hordein	2	**0.56***
Absorption	B:C hordein ratio	2	**-0.71****
Leaf N treatment plot 2	C hordein	3	**0.68****
Leaf N treatment plot 2	B hordein	3	**0.57***
Leaf N treatment plot 2	Total hordein	3	**0.67****

**** p≤0.01, * p≤0.05**

6.3.2.2 Across all nitrogen treatments

Absorption was significantly correlated (P ≤ 0.05) with B hordein and total hordein content (Table 6.4). There was a significant relationship (P ≤ 0.05) between germination at 24 h and the D:B hordein ratio and yield with the B:C ratio. Kernel N and protein content showed significant positive correlations with D and C hordeins, total hordein content (P ≤ 0.01) and D:B hordein ratio (P ≤ 0.05) and a negative correlation with the B:C hordein ratio (P ≤ 0.01).

Table 6.4. Significant correlations between malting quality traits and hordein fractions across all N treatments

Malting quality trait	Hordein trait	Correlation value
Absorption	B hordein	**0.25***
Absorption	Total hordein	**0.33***
Germination at 24 h	D:B hordein ratio	**0.32***
Yield	B:C hordein ratio	**0.35***
Kernel N and protein	D hordein	**0.36****
Kernel N and protein	C hordein	**0.52****
Kernel N and protein	Total hordein	**0.43****
Kernel N and protein	D:B hordein ratio	**0.30***
Kernel N and protein	B:C hordein ratio	**-0.50****

**** p≤0.01, * p≤0.05**

6.4 Discussion

Reverse phase-high performance liquid chromatography (RP-HPLC) analysis of storage proteins, in particular hordeins and glutens are used for cultivar identification and prediction of malting quality and bread making quality in barley and wheat respectively (Naeem and Sapirstein, 2007). In this study the different genotypes were identified by their distinct hordein patterns. There was no change in the composition of hordeins in response to timing of N application and to the environment. A study conducted by Wang *et al*. (2007) showed that hordeins were affected by N rate but less influenced by N application time.

In this study it was found that the two-row parent had the lowest total hordein content and the six-row progeny had the highest hordein content. This is expected as two-row barley has plumper grains than six-row barley (Ellis and Marshall, 1998) and plump kernels have a lower kernel N content (Gebhardt *et al*., 1993) thus, two-row barley will have lower total hordein content. The C hordein fraction was higher for the two-row progeny whilst the B hordein fraction was higher for the six-row parent. Six-row barley is used for animal feed and not for malt barley (Ellis and Marshall, 1998) and poor malting varieties have more B hordeins compared to C hordeins (Šimić *et al*., 2007).

Nitrogen was applied at different plant growth stages. Kernel N and protein content will be determined by the ability of the plant to use the available N applied at the different plant growth stages and to translocate the N from the vegetative leaves to the kernels (Savin *et al*., 2006). Hordeins account for about 60% of total kernel N content and their synthesis and accumulation in the kernel will therefore depend on the timing of N application (Šimić *et al*., 2007). A relationship existed between C and D hordeins at all N treatments. B hordeins were correlated with D and C hordeins at treatments one, two and three. These results show either that the timing of all N applications or the supply of additional N in the form of fertilizer may have influenced the production of all hordein fractions. The true effect of these relationships may be more apparent upon investigation of the effect of the different N treatments on the ratio of B:C and D:B hordeins.

Total hordein content was correlated with D:B hordein ratio at treatment one. This indicates that all the N should not be applied at planting as a high D:B hordein ratio decreases malt quality (Savin *et al*., 2006). A negative relationship existed between total hordein content and the B:C hordein ratio at treatments one, two and three. In this case applying all the N at planting or a split application at planting and at six leaf or flag leaf stage is also not recommended as a low B:C hordein ratio decreases malting quality (Savin *et al*., 2006). A relationship existed between D:B hordein ratio and D hordeins at all N treatments. This indicates that the timing of all N applications does not influence malting quality. A relationship also existed between B:C hordein ratio and D hordeins at treatments two and three.

The negative correlation between B:C hordein ratio and C at all N treatments suggests that the amount of B hordeins was greater than C hordeins which is not recommended as B hordeins are associated with decreased malting quality (Šimić *et al*., 2007) since total hordein content and B hordeins form the majority of total protein content in kernels (Howard *et al*., 1996). Marchylo *et al*. (1986) showed that poor malting varieties had more B hordeins compared to C hordeins (Šimić *et al*., 2007). The relationship between the B:C and D:B hordein ratio at treatment four indicates that the split application at planting and when 50% spikes are visible also does not influence malting quality. It can be concluded that the supply of additional N in the form of fertilizer and not the timing of all N applications influenced the production of all hordein fractions. These findings have also been reported by Howard *et al*. (1996) and Wang *et al*. (2007).

A negative relationship existed between kernel plumpness and B, C and D hordeins and total hordein content at treatment two. This relationship is expected as B hordeins comprise 70 - 80% of total hordein content and form the majority of total N content (Howard *et al*., 1996) and plumpness is associated with low kernel N content (Gebhardt *et al*., 1993). Baxter and Wainwright (1979) also found a negative relationship between B hordeins and malting quality.

Absorption was positively correlated with C hordeins and negatively correlated with the B:C hordein ratio at treatment two. The B and C hordeins form the majority of total hordein content hence a low B:C hordein ratio will facilitate water absorption and allow access of the hydrolytic enzymes to the starch substrate (Yan *et al*., 1999; Koning, 2006). Molina-Cano *et al*. (1995) suggested that C hordein had a positive effect on water uptake during malting. Leaf N was correlated with C and B hordeins and total hordein content when half the N was applied at planting and half at six-leaf stage. It is not known if all of the leaf N will be translocated to the kernels thus increasing kernel N and protein content and consequently total hordein content.

The relationship between malting quality and hordein fractions averaged across all N treatments was also determined. Absorption was correlated with B hordeins and total hordein content. Since B hordeins and total hordein content reflect total protein content in the kernels, absorption and malting quality may increase if B hordeins are present in a non-aggregated form thus enabling these hordeins to be degraded during malting (Howard *et al*., 1996; Celus *et al*., 2006). Relationships also existed between germination at 24 h and the D:B hordein ratio and with yield and the B:C hordein ratio. During germination D hordeins are degraded first, followed by B and C hordeins (Marchyllo *et al*., 1986). A possible explanation for this relationship may be that there are sufficient enzymes available to degrade the increasing amounts of D and B hordeins and thus germination rate also increases at 24 h. B:C hordein ratio is known to increase malting quality (Savin *et al*., 2006) and in this study may have played a role in increasing yield.

Kernel N and protein content was positively correlated with D and C hordeins, total hordein content and the D:B hordein ratio and negatively correlated with the B:C hordein

ratio. A similar negative relationship between kernel N and the B:C hordein ratio was found by Griffiths (1987), Molina-Cano *et al*. (2001) and Savin *et al*. (2006). These results further indicate that the timing of all N applications did not influence malting quality, since an increase in kernel N resulted in an increased D:B hordein ratio and a decreased B:C hordein ratio and thus reducing malting quality.

CHAPTER 7
General conclusions

Significant correlations were seen between leaf and kernel nitrogen (N) content for both the two- and six-row populations. These populations responded completely differently to N fertilizer application at the different plant growth development stages. The results indicated that N should be applied half at planting and half at the flag leaf stage for the two-row population and for the six-row population half at planting and the other half as a split application at the six-leaf and flag leaf stages. Although correlations were seen between leaf and kernel N for the two- and six-row populations, the single entries within a population responded differently over years and localities. It is therefore, impossible to sample leaves randomly from a population in an attempt to decide whether to apply N at a particular plant growth stage to obtain optimum N content in mature kernels, due to genetic variation and environmental influence. The environment should be taken into consideration when implementing fertilizer management systems and the response of different cultivars to N application will also have to be determined.

Timing of N application had an effect on all the malting quality traits i.e. kernel plumpness, yield, germination, absorption and kernel N of both the two- and six-row populations at the different environments. However, RP-HPLC results of hordein fractions showed that there was no change in the composition of hordeins in response to timing of N application and to the environment. A split N application at planting and six-leaf stage increased kernel plumpness of the two-row population at all environments compared to the six-row population which responded differently to all the N treatments at all environments. High yields were obtained for the two- and six-row populations at Vaalharts in 2006 when N was applied at planting and when 50% spikes were visible. However the lowest yields occurred in the following season possibly due to unfavourable environmental conditions.

Germination rate of the two-row population was increased by a split N application at planting and six-leaf stage at Vaalharts in 2006 and 2007 and at planting and flag leaf at Rietriver in 2007. For the six-row population, a split N application at planting and flag leaf increased the germination rate at all environments. The results also indicated that there

were no dormancy effects in the period of malt quality analysis. For absorption rate the best N treatments for two- and six-row populations were a split application between planting and flag leaf and between planting and six-leaf respectively at Vaalharts in 2007.

Kernel N was within the acceptable range of 1.5 - 1.95% for both populations only at Rietriver in 2007 when half the N was applied at planting and half at the six-leaf stage. At Vaalharts in 2006 a split application at planting and flag leaf stage resulted in low kernel N content, however, this did not occur the following season. A negative correlation existed between leaf N and kernel plumpness for the two-row population at treatment two over localities. This relationship strengthens the results obtained when a correlation was observed between leaf N and kernel N at treatment two for the two-row population over years and localities as low kernel N content is associated with plump kernels.

This study indicates that a practical N fertilizer strategy should include half the N application at planting (55kg/ha) for crop and tiller development and a split application at six-leaf and flag leaf to enhance kernel plumpness, germination, absorption and yield and maintain optimum kernel N for good malting quality. With further studies the ratio of N to be applied between the split application at the six-leaf and flag leaf stage needs to be optimised.

The two- and six-row entries within the doubled haploid population differed in malting quality across environments. In this study, genotypes contributed more to variation in the populations than the environment for both kernel plumpness and yield. However, for kernel N, the environment contributed more to variation than the genotypes. Germination differed greatly in six-row entries compared to two-row entries. The irregularities in kernel size of six-row barley may explain the variation in germination found in six-row entries compared to the two-row entries.

The different barley genotypes were identified by their distinct hordein patterns with RP-HPLC. The two-row parent had the lowest total hordein content and the six-row progeny had the highest hordein content. This is expected as two-row barley has plumper grains than six-row barley and plump kernels have a lower kernel N content thus, two-row barley will have lower total hordein content. The C hordein fraction was higher for the

two-row progeny whilst the B hordein fraction was higher for the six-row parent. Six-row barley is used for animal feed and not for malt barley and poor malting varieties have more B hordeins compared to C hordeins.

There were significant correlations between malting quality traits and hordein fractions. A negative relationship existed between kernel plumpness and B, C and D hordeins and total hordein content at treatment two. This relationship is expected as B hordeins comprise 70 - 80% of total hordein content and form the majority of total N content and plumpness is associated with low kernel N content. Absorption was positively correlated with C hordeins and negatively correlated with the B:C hordein ratio at treatment two. From literature it was found that C hordein had a positive effect on water uptake during malting.

Kernel N and protein content was positively correlated with D and C hordeins, total hordein content and the D:B hordein ratio and negatively correlated with the B:C hordein ratio. These results indicate that the timing of all N applications did not influence malting quality, since an increase in kernel N resulted in an increased D:B hordein ratio and a decreased B:C hordein ratio and thus reducing malting quality.

CHAPTER 8

Summary

Keywords: barley, doubled haploid population, malting quality, nitrogen applications, hordein fractions, RP-HPLC

The genotype effect of South African barley cultivars on malting quality under different nitrogen applications was determined by planting two- and six-row doubled haploid populations consisting of 7 and 67 lines respectively under irrigation at Vaalharts in 2006 and 2007 and at Rietriver in 2007. Three different nitrogen (N) treatments were applied to correlate the amount of leaf N to N in the kernel to implement a practical N fertilizer management system to obtain good malting quality. For treatment one, all of the fertilizer (110 kg/ha) was applied at planting. For treatments two and three, half of the fertilizer (55 kg/ha) was applied at planting while the other half (55 kg/ha) was applied at the six-leaf stage or when 50% of flag leaves were visible respectively. The best N application for the two-row population was treatment three compared to the six-row population which responded differently to all the N applications over years and localities. This study indicated that a practical N fertilizer strategy should include half the N application at planting (55kg/ha) for crop and tiller development and a split application of the other 55 kg/ha at six-leaf and flag leaf stage to enhance kernel plumpness, germination, absorption and yield and maintain optimum kernel N for good malting quality. Timing of N application had a significant effect on all the malting quality traits. However, RP-HPLC results of hordein fractions showed that there was no change in the composition of hordeins in response to timing of N application and to the environment. The two- and six-row entries within a doubled haploid population differed in malting quality across environments. Genotypes contributed more to variation in a population than the environment for both kernel plumpness and yield. However, for kernel N, the environment contributed more to variation than the genotypes. There were significant correlations between malting quality traits and hordein fractions. In particular, the negative correlation between kernel plumpness and total hordein content at treatment two. Total hordein content forms the majority of total kernel N content and plumpness is known to be associated with low kernel N content. The different barley genotypes were identified by their distinct hordein patterns with RP-HPLC. The C and B hordeins were

able to distinguish between two- and six-row genotypes. Averaged across all N treatments, kernel N and protein content was positively correlated with the D:B hordein ratio and negatively correlated with the B:C hordein ratio which indicates that malting quality was reduced.

Opsomming

Sleutelwoorde: gars, dubbeldhaploïede populasie, moutkwaliteit, stikstof behandelings, hordeïenfraksies, RP-HPLC

Die effek van genotipe op die moutkwaliteit van Suid-Afrikaanse garskultivars is onder besproeiing by Vaalharts (2006 en 2007) en Rietrivier (2007) bepaal. Daar is van verskillende stikstof behandelings (N) gebruik gemaak en twee- en ses-ry dubbeldhaploïede populasies, bestaande uit sewe en 67 lyne onderskeidelik, is hiervoor gebruik. Drie verskillende N-behandelings is toegedien om die korrelasie tussen blaar-N en N in die korrel te bepaal, met die oog op die daarstelling van 'n praktiese N-besmestingsriglyn om goeie moutgehalte daar te stel. Vir die eerste behandeling is al die bemesting (110 kg N/ha) met plant toegedien. Vir die tweede en derde behandelings is die helfte daarvan (55 kg N/ha) met plant toegedien en die ander helfte onderskeidelik by die ses-blaar stadium of wanneer die helfte van die vlagblare sigbaar was. Die mees voordelige N-behandeling vir die twee-ry gars was behandeling drie, in vergelyking met die ses-ry populasie wat anders gereageer het ten opsigte van al drie N-behandelings oor jare en lokaliteite. Uit hierdie studie is dit duidelik dat die helfte van die totale N met plant toegedien moet word vir gewas en halm-ontwikkeling. Latere toedienings van stikstof moet moontlik tussen ses-blaar en vlag-blaar stadium gedeel moet word om vetkorrel, ontkieming, absorbsie en opbrengs te bevorder met die behoud van optimale korrel N-inhoud vir goeie moutkwaliteit. Die tyd van N-toediening het 'n betekenisvolle effek op al die moutkwaliteitseienskappe gehad. RP-HPLC resultate het egter gewys dat daar geen verandering in die samestelling van hordeïenfraksies in reaksie op die tyd van N-toediening as gevolg van omgewing was nie. Die twee- en ses-ry inskrywings binne die dubbeldhaploïede populasie het oor omgewings vir moutkwaliteit verskil. Genotipe het meer as omgewing tot variasie binne 'n populasie vir beide vetkorrel en opbrengs bygedra. Ten opsigte van korrelstikstof het die omgewing egter meer bygedra as die genotipe. Daar was betekenisvolle interaksies tussen moutkwaliteitseieskappe en hordeïenfraksies. Die negatiewe korrelasie tussen vetkorrel en totale hordeïeninhoud by behandeling twee was veral opmerklik. Die totale hordeïene-inhoud maak die grootste deel van die totale korrel-N uit en vetkorrel word algemeen met 'n lae N-inhoud geassosieer. Die verskillende gars genotipes kan aan kenmerkende hordeïenpatrone met behulp van RP-HPLC uitgeken word. C en D hordeïene kan gebruik word om

tussen twee- en sesry genotipes te onderskei. Korrel-N en proteïeninhoud was oor alle N-behandelings positief met die D:B hordeïen verhouding en negatief met die B:C verhouding gekorreleer wat aandui dat moutkwaliteit benadeel word.

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