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Wheat (Triticum aestivum L) yields and protein profiles under varying plant densities,nitrogen doses and planting time in Botswana

Doctorate of Philosophy (PhD)

Crop Science (Agronomy)

By

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Wheat (*Triticum aestivum* L.) yields and protein profiles under varying plant densities, nitrogen doses and planting time in Botswana

A Thesis submitted to the Department of Crop and Soil Sciences in Partial Fulfilment of the Requirements for the Degree of Doctorate of Philosophy (PhD)

In

Crop Science (Agronomy)

By

PATRICK KELVEN MASHIQA (Student ID No. 200300205)

February, 2020

Main Supervisor: Prof. S. Ngwako

Co- Supervisor: Prof. F. Pule-Meulenberg

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CERTIFICATION

1. Prof. Samodimo Ngwako 25/02/2020

Main Supervisor's Name and Signature Date

2. Flora Pule-Meulenberg 08/03/2020

Co- Supervisor's Name and Signature Date

3. Dr Sedleseng Tshwenyane 25/02/2020

Head of Department's Name and Signature Date

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1. Prof. Samodimo Ngwako 25/02/2020

Main Supervisor's Name and Signature Date

2. Flora Pule-Meulenberg 08/03/2020

Co- Supervisor's Name and Signature Date

3. Dr Seeleseng Tshwenyane 25/02/2020

Head of Department's Name and Signature Date

4. prof. Khumoetsile Mmolawa 09/03/2020

Dean of Faculty's Name and Signature Date

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STATEMENT OF ORIGINALITY

The work contained in this thesis was compiled by the author at Botswana University of Agriculture and Natural Resources (BUAN) between August 2014 and July 2019. In exception of references made to, equations, formulae, procedures, etc., the work is original and it will not be submitted for the award of any other degree or diploma of any other University.

Author's Signature

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ABSTRACT

Field studies were conducted at the Botswana University of Agriculture and Natural Resources, Sebele, Gaborone, to determine the effects of planting densities, nitrogen application rate, and planting time, on two wheat genotypes during the winter planting seasons of 2015 and 2016, under irrigated conditions. The experiment was laid out in a Randomised Complete Block Design (RCBD) in a split-plot arrangement, with three replications. The main plot consisted of two wheat genotypes, Baviaans and 14SAWYT308, and subplots comprised of two plant density treatments, being (0.2m × 0.15m) 333,333 plants/ha and (0.2m × 0.2m) 250 000 plants/ha, sub-sub plots of five nitrogen doses, being 0 kg/ha (N_0), 50 kg/ha (N_{50}), 75 kg/ha (N_{75}), 125 kg/ha (N_{125}) and 200 kg/ha (N_{200}), and sub-sub-sub plots of two planting dates being 21st April and 05th May. Plant density of 333,333 plants/ha gave higher grain yield and enhanced most yield components than the 250 000 plants/ha density. Plant density of 333,333 plants/ha increased grain yield of wheat by 11.2% (2015) and 13.6% (2016) over the density of 250 000 plants/ha. Nitrogen input had positive effects on grain yield and yield components of wheat as it improved them. Grain yield increased up to 125 kg/ha of N, thereafter N did not increase yield further. Grain yield was increased by 64.0% (2015) and 66.1% (2016) at 125 kg/ha nitrogen rate over the control. Planting date of 21st April produced significantly more grain yield (about 23% higher) compared to 5th May date over the two years. The genotype 14SAWYT308 exhibited better yield and yield components characters than Baviaans in some treatment factors. It was found that wheat genotypes 14SAWYT308 and Baviaans produced maximum yield when planted on the 21st April at 333,333 plants/ha and nitrogen application rate of 125 kg/ha to maximize yield under South Eastern part of Botswana.

Pertaining to protein profiling, the presence or absence of protein bands revealed variations among sizes of protein bands. Grain from treatments showed the presence of proteins with molecular weight ranging from 17 kDa to 75 kDa, with protein bands of 22 kDa, 32 kDa, and 58 kDa identified to be most contributors to variability in treatments clustering. The results showed that the presence of glutenins of both high molecular weight and low molecular weight are likely to contain genes that supports good baking quality of flour, and also indicate a considerable amount of genetic diversity between studied treatments. With respect to protein profiling, the interaction of plant population, planting time and N at 125 kg/ha contributed more protein bands with low and high molecular weight glutenins and could produce wheat flour with good baking qualities. Grain protein bands seem to have been influenced mainly by addition of N, genotype and planting date rather than population.

Keywords: Plant density, nitrogen rate, planting date, protein profile

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List of Symbols and abbreviations

Symbol		Unit
APS	Ammonium per sulphate	ml
HI	harvest index	%
LAD	leaf area duration	days^{-1}
CGR	crop growth rate	g m^{-2} day^{-1}
NAR	net assimilation rate	g m^{-2} day^{-1}
EC	electrical conductivity	d sm^{-1}
NaOH	sodium hydroxide	%
K_2SO_4	potassium sulphate	grams
$CuSO_4$	copper sulphate	grams
H_2SO_4	sulphuric acid	ml
Tris	trisaminomethane	molar
HCl	Hydrochloric Acid	ml
TEMED	Tetramethyllethylenediamine	µl
g	grams	___
ha	hectare	___
kg	kilograms	___
LAI	leaf area index	___
LA	Leaf area	cm^2
m	meter	___
m^2	meter square	___
mm	millimetres	___
ns	non- significant	___

*	Significant at or below 0.05 ($p \leq 0.05$)	—
**	Significant at or below 0.01($p \leq 0.01$)	—
***	Significant at or below 0.001 ($p \leq 0.001$)	—
cm	centimetres	—
CV	coefficient of variation	%
DAS	days after sowing	—
N	Nitrogen	%
P	Phosphorus	mg/kg
K	Pottasium	mol/kg
Mg	Magnesium	mol/kg
Ca	Calcium	mol/kg
N_0	0 kg/ha nitrogen rate	kg/ha
N_{50}	50 kg/ha nitrogen rate	kg/ha
N_{75}	75 kg/ha nitrogen rate	kg/ha
N_{125}	125 kg/ha nitrogen rate	kg/ha
N_{200}	200 kg/ha nitrogen rate	kg/ha
Dt_1	21st April planting date	—
Dt_2	05th May planting date	—
Dn_1	260 000 plants/ha plant density	plants/ha
Dn_2	200 000 plants/ha plant density	plants/ha
kDa	Kilo Daltons	kda
PC	Principal Component	—
T	Time for observation	—
µL	micro Litre	µL
TDM	Total Dry Matter	—

NTSYC-Pc	Numerical Taxonomy and Multivariate Analysis	—
UPGMA	Unweighted Pair Group Method with Arithmetic mean	—
SDS-PAGE	Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis	—
DAR	Department of Agricultural Research	—

CHAPTER ONE

1.0 INTRODUCTION

Compared to the past, there are now more people to feed but the land for cultivation of arable crops is decreasing (FAOSTAT, 2016). This calls for cultivation of high value crops such as wheat (*Triticum aestivum* L.) to increase yield per unit area. Wheat is grown globally and it is the second most important cereal crop in the world behind maize and is a primary source of proteins for more than 4.5 billion people in ninety-four developing countries (Braun *et al.* 2010). Wheat provides more protein than any other cereal crops (Iqtidar *et al.* 2006). The world production of wheat in the 2016/17 season was 754.1 million tons, making it the third most produced cereal after maize and rice (FAOSTAT, 2016). The highest world producer was the European Union with 150 754 000 tonnes, followed by China with 129 000 000 tonnes, then India with 89 000 000 tonnes. In Africa, Egypt produces 8 100 000 tonnes, Morocco 5 800 000 tonnes, Ethiopia 4 250,000 tonnes, South Africa 1 750 000 tonnes, Zimbabwe 20 000 tonnes, and Malawi being the lowest producer at 2 000 tonnes (FAOSTAT, 2016). Consequently, other countries such as Botswana import wheat and for them to start producing wheat, agronomic studies are needed to determine varieties and optimal conditions it requires.

Wheat belongs to the genus *Triticum*, in the Poaceae family and it is widely adapted and grown in most regions across the globe (El-Nakhlawy *et al.* 2015). Normally wheat is grown under irrigation in the tropics and in lowlands away from the equator, while in the subtropics it is grown in winter months as winter wheat, with supplementary irrigation. Winter wheat is a long-day crop and its sensitivity to day length differs among genotypes, but most are quantitative long-day plants, implying that they flower earlier with long day length, but they do not require a particular day length to induce flowering. It thrives best in temperate zones and heading is delayed until the plant experiences a period of cold temperatures of 0 – $5^{O}C$ (Curtis, 2002).

Mean daily temperature for optimum growth and tillering is 15 – 20^{0}C, but can withstand low temperatures of down to -20^{0}C during the early stages of development (Curtis, 2002). Although a wide range of soils can support wheat growth, medium textured soils are most suitable, with pH range of 6 - 8 (Curtis, 2002). Like with other crops, fertilizer requirements vary from one country to another according to environmental conditions.

Wheat grains are ground into flour, and the greatest portion of the wheat flour is used for bread-making. Wheat grown in dry climates is generally the hard types, with a protein content of 11-15 percent and strong gluten. This type of wheat is suited for bread making. Wheat of humid areas is softer, with protein content of about 8 - 10 percent and weak gluten. This soft type of wheat produces flour suitable for cakes, cookies, and pastries. Wheat grain is also used in the manufacture of whiskey and beer, while wheat straw and bran are used as animal feed and in the manufacture of carpets, baskets and paper (Oyewole, 2016).

In Botswana studies on plant density, nitrogen fertilization and planting time of wheat are needed in order to improve the crop for its potential for economic diversification. Plant density is considered one of the key crop management practices and is accorded a high research priority (Sangoi *et al.* 2002) because it affects yield by influencing yield components such as number of spikelets per spike, number of grains per spikelet, and 100-grain mass. Ideal plant population per area depends on several factors such as water availability, soil fertility and row spacing, including density (Argenta *et al.* 2001). Consequently, there is value in defining relationships between density and wheat yield to establish optimum seeding rates for different regions. Ideally, plants should be arranged equidistantly from each other in the field, thereby equally sharing plant growth resources and eliminating competition. Plant density also is known to affect nitrogen use efficiency of wheat through increased root length density (Dai *et al.* 2014). Plant density determines the ability of the crop to capture resources and it is of particular importance in wheat production because it is under the farmer's control in most cropping systems.

In addition to plant density, and equally important is the soil fertility requirements, particularly the nitrogen nutrition of wheat. Most soils in Botswana have very low N contents (Pule-Meulenberg and Batisani, 2003). Universally, nitrogen is considered as the second most limiting factor in crop production and limits yield (Alam *et al.* 2007; Gooding and Davies, 1997). It is one of the major plant nutrients and is an essential constituent of all living cells. It forms part of chlorophyll, growth hormones, RNA, DNA and plant proteins (Andrews *et al.* 2013), and it plays a number of roles in plant growth and is involved in the synthesis and transfer of energy (Rana *et al.* 2017).

Nitrogen is applied in order to improve crop quality and to increase yield, and its application also contributes considerably to protein content (Ames *et al.* 2003), especially when fertilizer rates satisfy the requirements of both yield and protein formation (Woyema *et al.* 2012). Nitrogen is also one of the important factors that affect the quality and yield of wheat, thus, with less amount of nitrogen fertilizer the yield and quality of wheat will become compromised. In addition, unsuitable nitrogen doses lead to increased nitrate leaching, which contributes to eutrophication of surface waters (Myrbeck, 2014). Grain protein content of wheat is genetically controlled, but may vary widely for a cultivar or variety according to soil fertility, rainfall, location or temperature (Laubscher, 1981). Grain protein content affects bread-making quality characteristics of wheat. A high protein content is important in bread-making as high protein flour generally has a high loaf volume potential, high water absorption and produces loaves with good keeping quality (Finney *et al.* 1957).

In addition to grain protein content, the presence of gluten in wheat makes it unique (Kausar and Shahbaz, 2013). Gluten is a protein composite found in wheat and related grains like barley and rye. It gives elasticity to dough, helping it to rise and gives the product a chewy texture.

Optimum planting time is also one of the most crucial factors influencing wheat yield. It plays an important role in crop production as suitable weather conditions for wheat help in achievement of high grain yield (Ouda *et al.* 2005). Delayed sowing of wheat normally results in reduced individual plant growth and number of tillers (Nazir *et al.* 2004). In addition, delayed wheat sowing normally exposes the plants to higher temperatures in semi-arid conditions which reduce season length, resulting in reduced number of tillers, thus less grain yield (Eid *et al.* 1997; El- Marsafawy *et al.* 1998). On the other hand, planting wheat early under semi-arid conditions produced maximum plant height, 1000-grain mass, grains per spike, and grain yield (Qasim *et al.* 2008). Wheat is a new crop to Botswana and therefore it is important to determine the best time to grow it, since day length varies with season.

1.1 JUSTIFICATION

Wheat has been identified as one of the economic diversification crops to be planted in Botswana. The country has since decided to conduct research on winter wheat as a step towards its commercialization (DAR, 2012). In 2016 Botswana imported about 57 142 tonnes of wheat valued at P120.98 Million (UN Trading Economics, 2018).

Earlier research on evaluation of 15 wheat genotypes has identified some winter wheat genotypes that seemed to perform well under Botswana climatic conditions since 2006 (DAR, 2011).

Nitrogen, is one of the major constraints for arable agriculture in Botswana (Pule-Meulenburg and Batisani, 2003). Levels of N have been shown to be as low as 0.0008% in some soils around Gaborone (Pule-Meulenberg and Dakora, 2007). So far, information on the optimum amount of N required for various wheat genotypes is not known for Botswana conditions. Such data are required because wheat yield and grain protein content depend on the amount of nitrogen applied to the plant. Protein content is an important issue in wheat because the price that a producer receives is determined by grain protein content, and it has a significant economic consequence for wheat producer (Orloff *et al.* 2012). Grain protein content is also important in bread-making as high levels of proteins in wheat normally lead to bread of high quality characteristics. Therefore, it is imperative to apply optimum level of nitrogen to wheat in order to attain highest yield at the desired protein levels.

Besides N, plant density also affects the yield of winter wheat and optimum plant densities vary greatly between areas, climatic conditions, soil and varieties. Since cultivars genetically differ for yield components, individual cultivars need to be tested at different plant densities to determine their optimum rate. There are no recommended planting densities of wheat for Botswana conditions.

It is important to determine the optimum time to plant wheat as this will ensure the right conditions which may ultimately result in yield improvement. Therefore, optimum planting date is needed to produce good yields in wheat as this ascertains suitable growth conditions which optimize wheat grain yield by providing suitable agro-climatic conditions at each growth stage. Research on how planting date affects wheat in Botswana has not been done. As the country moves towards commercialization of agriculture, research on various aspects of wheat is necessary. Therefore, this study examines how plant density, nitrogen application and planting date interact and affect wheat genotypes grown in Botswana. Knowing the yield and yield components of wheat, its protein content and ultimately the profile of the protein with respect to the ratio of low molecular to high molecular glutenins will assist decision making before release of the genotypes to farmers as local varieties.

In a preliminary wheat study (DAR, 2011), the two wheat genotypes Baviaans and 14SAWYT308 were evaluated for grain yield under 3 planting densities of 250 000 plants/ha (20cm *20cm), 333 333 plants/ha (20cm *15cm) and 500 000 plants/ha (20cm *10cm) and 5 planting time (14 April, 21 April, 28 April, 5 May and 12 May). Both genotypes gave promising grain yield results under 333 333 plants/ha density and planting date of 21st April. Therefore to reduce experiment size for the current study the 2 genotypes were evaluated under 2 plant densities, 2 planting dates in addition to 5 nitrogen regimes, in-order to test the genotypes for more agronomic and physiological parameters.

1.2 AIM AND OBJECTIVES

The aim of this research was to investigate the influence of plant density, nitrogen rate, and planting date on growth, yield, and yield components, the protein content as well as the molecular profile of the grain proteins of two wheat genotypes.

The specific objectives of the study were:

1. To determine the effect of plant density on yield and yield components of wheat genotypes.
2. To determine the effect of nitrogen applied at different rates on wheat genotypes under field conditions.
3. To determine the effect of planting date on yield and yield components of wheat genotypes.
4. To evaluate the protein profiles of wheat genotypes for prediction of their bread making qualities.

1.3 HYPOTHESIS

H_{A1}: Plant density has a significant effect on yield and yield components of wheat genotypes.

H_{O1}: Plant density has no significant effect on yield and yield components of wheat genotypes.

H_{A2}: Nitrogen application rate has a significant effect on yield and yield components of wheat genotypes.

H_{O2}: Nitrogen application rate has no significant effect on yield and yield components of wheat genotypes.

H_{A3}: Planting date has a significant effect on yield and yield components of wheat genotypes.

H_{03}: Planting date has no significant effect on yield and yield components of wheat genotypes.

H_{A4}: Protein profiles has a significant effect on wheat bread making quality.

H_{04}: Protein profiles has no significant effect on wheat bread making quality.

CHAPTER TWO

2.0 LITERATURE REVIEW

There are many components of production technology which can significantly affect grain yield and yield components of wheat, among them are plant density, nitrogen fertilization rate, and planting date. The grain yield of any wheat variety is dependent on its yield components as Sheron *et al.* (1986) observed that spike length, plant height and grains per spike were directly related to grain yield. In Botswana it is not possible to discuss wheat yields because it is a new crop whose suitability for growth is not yet fully know. Thus this study is among the few that attempt to establish factors such as varieties suitable for local conditions, planting densities, rate of N application and suitable time for planting in this country.

2.1 Effect of plant density on wheat

Generally, increases or decreases in plant density in crops can either have a negative or a positive effect on the productivity of the crop. Whether a crop can tolerate high plant density often depends on many factors such as genotype, soil type and general environmental conditions. For example, Ghaffar (2013) demonstrated that some wheat varieties produced higher grain yields at higher plant densities. Hiltbrunner *et al.* (2007) reported that the optimum planting densities in wheat are the key to achieving maximum yield. It is believed that for each cropping system and each cultivar, a specific and desirable density is required.

With respect to crop phenology, plant density has been reported to significantly influence number of days to emergence of wheat. According to Hameed *et al.* (2003), crops sown at higher density took more days to emerge than those at lower density, and this was attributed to stronger competition for food and moisture whereas these requirements were fulfilled at lower density. Khan *et al.* (2017) reported that an increase in plant density led to increased number days to plant maturity. The rate of plant development may have slowed down due to more mpetition for water and nutrients under higher density, thus resulting in more days to reach ysiological maturity.

rmally in wheat, grain yield is influenced by yield components, which include number spikelet per spikes, number of grains per spike and 1000- grain mass. Number of grains r spike depends on the length of spike and it is determined by genetic make -up and vironmental factors prevailing during the growth period. For instance, Sulieman (2010) ported that an increase in density resulted in a slight increment in the heights of the plants, d it was because of variable environmental conditions and genetic makeup of the genotypes ed in the studies.

wheat, studies conducted in Iran (Naseri *et al.* 2012) and Pakistan (Ghaffar, 2013; Iqbal, 10) indicated that increasing the plant density of various wheat varieties resulted in increased elds. In a study conducted by Ali *et al.* (2016) densely populated wheat plants gave gnificantly higher grain yield than lower density plants, and was mainly attributed to the creased density of spike per unit area. Although higher density treatment had lower 1000 rnel mass than the lower density treatment, the difference observed was slight and mpensated by increased tiller per unit area. Under higher density the narrow spacing causes ore even spatial plant distribution, increased crop ground cover, leaf area index, light terception and dry matter. Thus, narrow spacing also decreases weed population and reduces oil evaporation (Chen *et al.* 2010; Drews *et al.* 2009), hence increased grain yield. In addition, aximum grain yield under increased density may be attributed to improvement in number of illers, number of spikelets per spikes and one thousand grain mass. In a favourable nvironment there is a uniform yield due to regular tiller formation and to the distribution f photosynthates, which contribute to grain yield (Rickman *et al.* 1983). Improvement in ht interception during critical period for grain set may also increase yields at higher ısities (Andrade *et al.* 2002). A number of researchers have also reported that narrow row ıcing (higher density) gave better yield in wheat than wider row spacing (Chen *et al.* 2008; ınson *et al.* 1988; Joseph *et al.* 1985; Marshall and Ohm, 1987; Mohammadi *et al.* 2012; mpkins *et al.* 1991).

e above literature show that yield and yield components increase with increased density. ıwever, not all studies conclude that increasing plant density results in elevated yields or ated parameters. For instance, Hussein *et al.* (2018) who compared seeding rate of 75, 100, d 125 kg/ha in the semi-arid region of Pakistan, showed that there were no significant ferences in grain yield and yield components of wheat. In addition, Penuar and Sirrbie 989) reported a non-significant effect of plant density on number of days to emergence of ıize under similar semi-arid conditions. Other investigators also reported no significant fference in yield and yield components of wheat as affected by density (McLeod *et al.* 1996). contrast, some researchers observed reduced yield and/or yield components as density creased (Fromme *et al.* 2012; Hiltbrunner *et al.* 2005; Lafond, 1994; Lafond and Gan, 1999). oosavi *et al.* (2013) investigated the effect of plant density on yield and yield components of ain sorghum under climatic conditions of Sistas, Iran, and sub-plots comprised of four plant ınsity levels of 12, 15, 25, and 50 plants m^{-2}. It was found that increase in plant density up to) plants m^{-2} resulted in a significant loss of 1000-grain mass, grain number per panicle and ırvest index.

ccording to Li *et al.* (2016) number of grains per spike, grain mass and grain yield decreased ith increased density due to seed setting characteristics of the varieties used in the study. long the same line, Andrade *et al.* (1993) reported a sharp decline in kernel number per plant ith as plant density increased and attributed the response to a decrease in photosynthetic rate er plant and hence plant growth rate. Similar observations were shared by Tokatlisdis and utroubas (2004) who also reported that under higher plant density the reduced assimilates ›ply caused abortion of kernels. Similarly, Singh (2013) reported that a higher density ulted in reduced number of spikelets per spike, and it was attributed to smaller spacing which reased competition between plants for nutrients, water, space and light interception. rthermore, Ayalew *et al.* (2017) observed that higher plant density resulted in reduced mber of tillers in wheat. When seed rate is higher it results in narrow free space which limit : tillering capacity of the plant. The higher seed rate might have increased competition for ıce, thereby resulting in lesser number of tillers per plant (Baloch *et al.* 2012). According to : results of Modarresi *et al.* (2002) the reduction in the mass of 1000 seeds in high densities ıy be due to the superiority of vegetative organs in competing with reproductive organs. nilar findings of 1000 seeds mass reduction in high densities was reported by Gardner)07).

2 Effect of nitrogen on wheat

trogen fertilizer application plays a significant role in improving soil fertility and crop oductivity. According to Brady and Weil (2002) high N favours the conversion of rbohydrates into proteins, which in turn promotes the formation of protoplasm, which is the ıilding substances from which materials are made. Since it is a necessary component of all oteins, N is involved in all plant growth processes. In wheat, nitrogen is known to affect the ıenological stages, yield, and yield components.

ıd and Arora, (1990) in a field experiment reported a greater number of days to emergence, ıthesis, and physiological maturity as nitrogen fertiliser was increased. The increase in days ı anthesis as N was increased was attributed to increased uptake of nutrients during the llering stage, which might have prolonged vegetative growth, thus increased days to anthesis.

The delay in maturity of wheat when N levels was increased up to 160 kg/ha of N was ascribed to increased grain filling period due to more accumulation of photosynthates.

Nitrogen fertilizer is known to affect the number of tillers m^{-2}, number of spikelets per spike, number of grains per spike, spike length and 1000- grain mass (Ali *et al.* 2000). Generally an increase in N application up to an optimum level results in increased yield and yield parameters. In a field experiment, application of N fertiliser increased number of spikelets per spike of wheat (Mosanaei *et al.* 2017). The researchers reported that the increase in nitrogen consumption increased the number of spikes per unit area, which increased vegetative growth and, consequently, increased the amount of tillering due to nitrogen consumption. In such a situation, the number of fertilized tillers per unit area increases and the number of spikes per unit area also increased (Nourmohammadi *et al.* 2010).

Gill and Ali (1985) reported that split application of nitrogen at sowing and tillering stimulated the formation of tillers, and Picciurro *et al.* (1972) attributed the increased tiller production to high utilisation of N in the early stages of growth. Likewise, Ali *et al.* (2011) and Sud and Arora (1990) reported a significant increase in number of tillers m^{-2} as N increased over control. In wheat, N fertiliser is normally used to manipulate number of tillers as well as supply the nutritional requirements of the crop. Wheat plants grown with enough supply of N grow rapidly due to rapid conversion of synthesized carbohydrates into protein and consequently increase the number and size of growing cells, resulting ultimately in increased number of tillers (Singh and Agarwal, 2001).

The effect of nitrogen on growth and yield components of wheat was studied by Ali *et al.* (2011) in Sargodha, Pakistan. The wheat crop was given nitrogen doses of 0, 80, 130, and 180 kg/ha using the variety Sehar-2006. According to the results the number of grain per spike was significantly increased by increase in nitrogen levels compared to control. The significant increase in number of grains per spike could be due to enhanced tillering, photosynthetic area and increased sink size in presence of adequate nitrogen (Tiwari *et al.* 2017). Similar research findings were reported by Patel *et al.* (1994) and Singh and Singh (1989).

Studies conducted by Shah *et al.* (2018) reported a significant impact of nitrogen on 1000-grain mass with higher means observed at maximum dose of N. This may be associated with prolonged availability of nitrogen nutrient to wheat. The transfer of photosynthetic material from the source (leaf) to the sink (seed) can be a reason to increase the mass of 1000 seeds (Mosanaei *et al.* 2017). Additionally, in the investigation by Sadeghi and Kazemeini (2011), increasing the amount of nitrogen application also increased the mass of 1000-seed in barley varieties. Since nitrogen fertilizer increases dry matter production and leaf area, barley grain also became heavier with increasing nitrogen application. Other researchers who reported an increased mass with increased N fertiliser included Kumar *et al.* (2000) and Sharma *et al.* (2000).

According to the results of Gwal *et al.* (1999), plant height of wheat increased significantly with increase in N rates. Nitrogen can increase wheat growth by affecting cell division as well as assisting in the absorption of nutrient elements by the plant; hence the increase in nitrogen can increase the plant height of wheat (Asadie *et al.* 2013). Adequate supply of N can stimulate increase in activity of meristemic cells and cell elongation of internodes resulting in higher growth rate of stem, in turn promoting plant height of wheat (Mattas *et al.* 2011). In addition increase in N application can result in increased protein content of the cells, which ultimately expand in size; as a result the leaf area enlarges, followed by photosynthetic activity and consequently increased plant height (Wysocki *et al.* 2007). The results were supported by the work of Jan *et al.* (2002); Mosanaei *et al.* (2017), and Sud and Arora (1990) who observed an increase in plant height due to nitrogen fertilization.

Patra and Ray (2018) studied different levels of nitrogen (120, 135, 150 and 165kg/ha) on wheat in India. The investigators concluded that increased in straw yield with increased N was probably due to increased growth parameters like plant height, number of tillers and dry matter production. Similar results where corroborated by Narolia *et al.* (2016) and Pandey *et al.* (2014).

According to results of Mosanaei *et al.* (2017), nitrogen increased biological yield of wheat. The effects of the application of the nitrogen fertilizer on biological yield were attributed to increased plant height, seed yield, leaf dry mass, stem dry mass, and tiller number. McDonald (2002) also reported that increased nitrogen application significantly increased dry matter of wheat.

The results of Shah *et al.* (2018) showed that a higher harvest index was recorded with increased nitrogen rate. The phenomenon was associated with more accumulation of nitrogen toward vegetative as well as reproductive growth of crop. The increase in harvest index indicates that the plant is more able to transfer and allocate more material to the seed, and is one of the indicators used to evaluate the efficiency of the distribution of the dry matter of the agricultural crops. In grains, the increase in biomass has reached its final limit, hence the increase in seed yield through the allocation of more photosynthetic materials to the sink (seeds) is possible, which may result in harvest index significantly increasing (Krishnan *et al.* 2003).

Grain yield is the end product of many yield-contributing components, physiological and morphological processes taking place in plants during growth and development. In a field investigation, Tayebih (2011), indicated that the different N rates (120, 240 and 360 kg/ha) have a significant effect on grain yield increment (46% at N120, 72% at N240, and 78% at

N360) compared to control. The increase in grain yield was attributed to increase in the yield components as the level of nitrogen was increased.

Haileselassie *et al.* (2014) undertook an on-farm field experiment to assess the effects of nitrogen on yield and yield components, and protein content of bread wheat in Ethiopia in two fields. During the growing season the area received an annual mean maximum temperature of 24 ^{O}C and a mean minimum temperature of 7.7 ^{O}C. Treatments included five nitrogen levels of 0, 46, 69, 92, and 138 kg/ha of N. Results revealed that grain yield of wheat in field 1 and field 2 significantly increased by 46% and 15% respectively, at 46 kg/ha of N application rate than the control. Nitrogen fertilization increased straw yield in experimental field 1. Generally nitrogen has greatest effect on photosynthesis, therefore, seed yield increased with nitrogen application. Nitrogen could have increased vegetative growth, which resulted in more leaf area to intercept more photosynthetically active radiation, which resulted in more grain filling, hence increased seed yield (Mosanaei *et al.* 2017).Additionally, the higher grain yield with maximum level of N could be attributed to abundant availability of plant nutrients, resulting in more tillers, longer spike, and more grains per spike, which ultimately led to increased grain yield.

Along the same line, Ali *et al.* (2011) reported that applying N to a wheat crop also resulted in an increase in grain yield. Nitrogen increased the biomass production and increases the possibility of transmission of photosynthetic materials, producing more seeds per spike and better grain filling after flowering, which will increase grain yield (Shanggan *et al.* 2000). Many other researchers observed an increase in grain yield over control as nitrogen was added to wheat crop (Ardell *et al.* 2001; Camara *et al.* 2003; Galieni *et al.* 2016; Gorjanovic *et al.* 2010; Hasan *et al.* 2016; Kibe *et al.* 2006; Ortega *et al.* 2000).

Interestingly, some researchers found that nitrogen application had no significant effect on wheat measured attributes. While nitrogen addition to crops is vital, the crops tolerate it to a certain level where its impact remains significant. For example, Maqsood *et al.* (2000) reported that increasing nitrogen rates beyond 100 kg/ha did not significantly increase grain yield of wheat. Similar results were shared by Singh and Uttam (1992) who reported that grain yield increased significantly only up to 120 kg/ha of N. Similarly, applying N to wheat was observed to have no significant variation in 1000- grain mass (Khokhar *et al.* (1985; Sharma and Tripathi 1999).

Khairna *et al.* (2018) planted wheat at an experimental research farm in Hol to assess the performance of wheat under N-levels of 40, 60, and 80 kg/ha. Results showed that nitrogen did not significantly influence number of days to physiological maturity. Along the same line, Sud and Arora (1990) found out that application of N had no significant effect on 1000-grain mass and number of grains per spike of wheat.

Relationship between N application and grain protein

Grain protein content and composition is affected by both genotype and the environment in which the crop is grown. Protein content in wheat grain can be increased by increasing level of nitrogen fertilizer application (Uhlen *et al.* 2004). It is estimated that about 30 to 40% of the total N-fertilisers applied are actually harvested in grain (Belete *et al.* 2018). Therefore application of the appropriate rate of N fertiliser is considered to be a primary means of increasing wheat grain yield in improving N uptake and use efficiency and consequently nitrogen harvest index in grains (Fageria, 2014). Nitrogen application has been found to increase grain protein content in wheat, for instance, in the work of Belete *et al.* (2018), 360 kg/ha of N significantly increased grain protein of wheat over control in both years of study.

However, Zhang *et al.* (2017) reported a slightly lower, but non-significantly value (15.34%) of protein content when 300 kg/ha of nitrogen was applied to wheat, compared to a higher protein content value (15.40%) at 240 kg/ha of N. However, not all researchers found increased grain protein with higher levels of N. For instance, in a field experiment Abedi *et al.* (2011) investigated the effect of nitrogen rate and timing on yield and grain protein content of wheat using N rates of 0, 120, 240 and 360 kg/ha. Their results showed that grain protein content was significantly affected by N rates, and highest grain protein content was recorded at 240 kg/ha, it reduced at the N dose of 360 kg/ha. In another experiment, Campillo et al. (2010) applied nitrogen to wheat at 0, 150, 200, 250 and 300 kg/ha. According to the results grain protein content increased significantly with values of 8.19, 9.62, 10.29 and 11.89 for control, 150, 200, and 250 kg/ha respectively, but decreased to 11.30% when 300 kg/ha of N was added.

Grain protein content and bread making quality of wheat

Proteins are the most important component of wheat grains governing the rheological properties of flour and are closely associated with end-use quality (Zhao *et al.* 2010). Grain protein content, particularly glutenin content, has been positively correlated with Zeleny sedimentation value (SV) and stability time, and is thus regarded as an important index for wheat quality in bread making (Shi *et al.* 2005).

Wheat quality depends on the presence of certain alleles at loci, which are responsible for end product quality (Bagulho *et al.* 2015). Thus, if a certain genotype possess some specific allele combination at crucial loci, then it is possible to exhibit valuable qualitative trait in terms of end product quality (Varzakas *et al.* 2014). However, production of a cultivar conferring improved quality also require a growing environment that favours expression of this genetic potential in order for this to lead to the eventual production of high quality grain (Yong *et al.*

2004). Grain protein content is the major component that affects wheat quality. Therefore grain protein content is influenced strongly by environmental conditions and crop management practices, while protein quality is genetically controlled (Cornish *et al.* 1991; Mikhaylenko *et al.* 2000; Payne *et al.* 1987).

2.3 Effect of planting date on wheat

Planting date is one of the most important agronomic factors involved in producing high yielding crops like wheat. The crop responds differently to time of planting as its growth stages from time of emergence to maturity are affected by the prevailing environment. The physiological functions and growth stages are severely affected by temperature which decides the duration of the life cycle of wheat plant, which ultimately affects yield components and grain yield positively or negatively.

With regards to crop phenology, a study by Sud and Arora, (1990) revealed that later planting took more days to emerge and they attributed that to lower temperatures. Growth of late planted wheat is generally slow because of low temperature (Joshi *et al.* 1992). Normally when temperatures go down the process of germination becomes slow and ultimately the crown root initiation stage is prolonged, resulting in delayed emergence.

Number of days to anthesis were also reported to be less for later planted wheat crop, and this was attributed to quicker vegetative growth, which took shorter time, and resulting in quicker anthesis (Sud and Arora, 1990). With regards to crop maturity, Singh and Dhaliwal (2000) reported delayed maturity in earlier sown crops due to increase in temperature and faster wind velocity. In addition, Ouda *et al.* (2005) also reported that crops sown later matured earlier than those planted earlier. Sometimes dry winds and heat stress during grain filling can result in quickening of crop maturity.

El-Nakhlawy *et al.* (2015) in a field experiment evaluated wheat genotypes under arid conditions of Jeddah in Saudi Arabia. The results showed that most studied parameters were favoured by earlier planting date as number of tillers increased by 17 %. The increase in wheat yield and its components, including number of tillers, with earlier planting was attributed to exposure of plants to lower temperatures, which increased season length (Suileiman *et al.* 2014), hence more photosynthates accumulated to form grain. Fateminick and Azizi (2016) and Tahir *et al.* (2009) also showed that early planted wheat produced significantly increased number of tillers m^{-2} than later sown crops. Along the same line Sud and Arora (1990) attributed heavier grains in earlier planted crops to suitable and longer environmental conditions supporting vegetative growth, which resulted in the synthesis and translocation of maximum photosynthesis to the grains, thus increased grain mass. In addition, more number of grains per spike were achieved when sowing wheat early, and attributed the results to longer growing period, which resulted in more production of photosynthates to form grains (Munsif *et al.* 2016).

According to results reported by Sokoto and Singh (2013), number of spikelets per spike decreased with delay in sowing. Decrease in number of spikelets per spike was thought to be as a result of delayed sowing in late sown plants, coupled with higher temperature experienced in the months of summer, photosynthesis had a broad temperature optimum from 20°C to 30°C with photosynthesis and translocation declining rapidly at temperatures above 30°C (Wardlaw, 1974). Ishag (1993); Shafiq (2004) also observed that delayed sowing decreased the number of spikelets per spike. Cereals respond significantly to varying environmental features as their growth and grain development is highly temperature and moisture dependent (Eslami *et al.* 2014).

According to the results of Munsif *et al.* (2016), earlier planted crops were taller than those of later planting dates. This could be attributed to availability of long duration for maximum vegetative growth. In addition, early sowing might provide better growing conditions as optimal temperature and solar radiation provide taller plants (Fateminick and Azizi, 2016; Irfaq *et al.* 2005; Kamrozzaman *et al.* 2016; Qasim *et al.* 2008; Razzaq *et al.* 1986; Shafiq, 2004; Shahzad *et al.* 2002; Sud and Arora, 1990; Tahir *et al.* 2009).

On the other hand, late planting can have a negative effect on yield and yield components of wheat crops. In a field experiment Suleiman *et al.* (2014) reported reduced grain yield in late planted wheat. They attributed the reduction in wheat yield and its components with delayed planting date to exposure of plants to high temperature, which decreased season length. It has been documented that planting at inappropriate times may cause drastic reduction in wheat yield (Sattar *et al.* 2015). Along the same line, other investigators observed a high reduction in 1000-grain mass and grain yield when sowing was delayed (Subhan *et al.* 2004; Hussain, 2007). Ansary *et al.* 1989; Fateminick and Azizi 2016; Sharma *et al.* 2006) reported that late sowing of wheat significantly decreased germination percentage. The delay in sowing suppressed the yield, caused by reduction in the yield contributing traits like number of tillers, number of grains per spike and grain yield. Likewise, Shirinzadeh *et al.* (2017) studied the effect of planting date on growth periods, yield, and yield components of some bread wheat cultivars in Parsabad Moghan in Iran and reported lowest grain yield and other yield components (1000-grain mass, number of grains per spike, biological yield, and plant height) in late planted wheat. Reduction in grain yield at later planting date was attributed to shortening of grain filling period, reduction in number of seeds, increase in temperature during grain filling period and 1000-grain mass. Their results were supported by (Cassim, 2008; Kumar *et al.* 2013; Zia *et al.* 2014).

Some researchers did not find any significant difference in yield or yield components being affected by planting date. According to results reported by Kamrozzaman *et al.* (2016), earlier planted wheat did not significantly differ compared to a later planting one.

Several factors influence spike traits, and sowing time is one of them. Spike length relates directly to number of grains per spike and finally to yield (Shahzad *et al.* 2007). However, Munsif *et al.* (2016) found that sowing dates did not significantly influence spike length. In another investigation Tahir *et al.* (2009) also did not find a significant difference in number of grains per spike as affected by sowing date.

Wheat planting date has been reported in literature to significantly influence harvest index (Kamrozzaman *et al.* 2016). The researchers reported lowest value of harvest index (45.35%) from the later planting date. Harvest index was said to have decreased markedly due to late planting, and it indicated that grain yield was reduced more than total biomass under late sowing conditions.

Relationships between genotype, nitrogen, planting time and leaf area index, photosynthesis and crop yield in wheat

Leaf area index (LAI) is an important parameter in wheat because leaves help in absorbing and assimilating carbon dioxide, intercepting necessary Photosynthetically Active Radiation (PAR) necessary for photosynthesis (Addai and Alimiyaw, 2015). Therefore LAI is an important indicator of PAR interception and energy conversion which are contributing factor to plant growth and yield formation. The relationship between LAI and yield is complex and will vary with crop type and different stages of a plant. In wheat, Bavec *et al.* (2007) reported increased LAI with increased density, and in addition interaction of variety and planting density also produced significant LAI in wheat. In another research Rahman *et al.* (2000) and Serrano *et al.* (2000) reported increased LAI with higher rates of nitrogen.

In the work of Rehman *et al.* (2010) LAI increased with the application of highest level of N (80 kg/ha), recording maximum LAI of 2.5. N fertiliser application to wheat during early stages of development greatly increased leaf area by delaying leaf senescence, sustained leaf photosynthesis and extended leaf area duration, which ultimately resulted in increased LAI compared to control (Zhang *et al.* 1998). In another investigation LAI increased significantly with increasing levels of N from 0 to 90 kg/ha at all growth stages of wheat (Mishra *et al.* 1998). The increase in LAI results in more canopy available to intercept photosynthetically active radiation which plays a key role in manufacturing of assimilates, hence increased grain yield. Increasing nitrogen level from 0 to 120 kg/ha significantly increased LAI, crop growth rate and ultimately grain yield of wheat (Sabry *et al.* 1999).

With regards to planting time, LAI is normally influenced due to temperature effect. Late planting, when temperatures are higher resulted in faster development of wheat, which led to early senescence and reducing photosynthetic leaf area, which ultimately reduced canopy during reproductive stage and major yield loss (Kumari *et al.* 2007). In addition late planting when temperatures are higher caused reduced grain number and duration of grain filling, hence reduced grain yield (Barlow *et al.* 2015).

According to the above literature, response of wheat to different planting densities, nitrogen regime and planting dates differed. For instance, increased density was reported to increase yield and yield components, while the opposite was true for the lower density. The results were varying for different agro-ecological conditions and different genotypes responded differently. In Botswana, however, no literature pertaining to such studies in wheat has been generated, therefore it leaves a gap to be filled and to explore these three factors under Botswana conditions in order to improve yield in wheat.

2.4 Protein profiling in wheat

Wheat grains are ground into flour, and most wheat flour is used for bread- making. One important factor for bread quality wheat is its protein content. Wheat grown in dry climates is generally the hard type, with a protein content of 11-15 percent and strong gluten. This type of wheat is suited for make bread. Wheat of humid areas is softer, with protein content of about 8 – 10 percent and weak gluten. This soft type of wheat produces flour suitable for cakes, cookies, and pastries. Therefore it can be important to determine not only the protein content in wheat seed, but also to profile the present type of proteins, the gluten.

Wheat grain protein can be divided into two groups; Non-gluten proteins (albumin and globulins) and gluten proteins (gliadins and glutenins). Gluten protein subunits are divided into High Molecular Weight Glutenin (HMW-GS) and Low Molecular Weight Glutenins (LMW-GS), and the gluten, is responsible for bread making quality (Branlard & Dardevet, 1985). They are one of the important flour quality determinants because they are responsible for dough extensibility and elasticity of wheat flour, which determine the processing qualities of a wide range of wheat products (Rasheed *et al.* 2014). Gluten also permits the retention of gas bubbles during baking of dough to give open textured and pleasant eating product (Manley *et al.* 2011), hence improved quality. The amount of gluten in wheat is normally influenced by the environment and the grain protein content, but however, influence of genotype is usually dominant for qualitative properties of gluten (Simic *et al.* 2006). SDS-PAGE method is used to separate gluten protein subunits into High Molecular Weight Glutenin (HMW-GS) and Low Molecular Weight Glutenins (LMW-GS). It has been reported that HMW-GS subunits have the largest effect on bread making quality even though they constitute only 10% of the total storage proteins as compared to LMW-GS, which contributes with 40% (Kaya and Akcura, 2014). The HMW-GSs described as Glu-A1, Glu-B1, and Glu-D1 are encoded by multi-allelic genes located on the long arms of chromosomes 1A, 1B and 1D respectively (Payne et al., 1984). It has been reported that HMW-GSs are encoded by three loci, Glu-A1, Glu-B1, and Glu-D1 (Payne & Lawrence, 1983). LMW-GSs are encoded by three loci, Glu-A3, Glu-B3, and Glu-D3 (Gupta & Shepherd, 1990).

HMW-GS and LMW-GS are shown by the protein bands on the gel after the separation of seed storage protein by electrophoresis on the basis of molecular weight, and the results can be used for genetic analysis and applicable in breeding purposes (Hnapek *et al.* 2014). Protein profiling may assist in knowing the type of proteins present in wheat seed/treatment, and such information can be used to determine the quality and use of the wheat in bread making.

How bread making quality is affected by genotype, density, nitrogen and planting date

One well known effect of nitrogen application is the increase in grain protein content (Xue *et al.* 2019). Grain protein concentration is widely is widely used as the main parameter in evaluating baking and bread making quality of wheat products, and higher price is usually achieved with high protein bread wheat (Xue *et al.* 2019). However, the baking quality is complex, and not only determined by grain protein concentration, but also its composition (Chaudhary *et al.* 2016).

Gluten proteins (gliadins and glutenins) play a major role in determining the baking quality of wheat flour as gliadins mainly contribute to dough viscosity and extensibility, while glutenins are vital for dough strength and elasticity (Wieser, 2007). Some studies have demonstrated that addition of N increased grain protein mainly through enhanced gliadins and glutenins, as well as the increased proportion of HMW-GS and w-gliadins while decreased proportion of LMW-GS (Wiser and Seilmeier, 1998). HMW-GS are of particular interest in bread wheat because of their large influence on the rheological properties of dough (Vawser and Cornish, 2004) and their involvement in the genotype by environment interaction particularly regarding gluten strength and extensibility (Panozzo and Eagles, 2000; Hristo *et al.* 2010).

Therefore, split N application is supposed to be an effective way in improving baking quality of wheat (Xue et al. 2019). In another study Xue *et al.* (2016) added split N to two winter wheat cultivars Tobak JB Asano and demonstrated that it improved bread loaf volume of both wheat cultivars mainly through the proportionally increased gliadin and glutenin fractions as well as certain HMW-GS as fractionated by SDS-PAGE. In addition, a study by Kaur *et al.* 2016 showed that N application significantly affected protein profile, textural and pasting properties of different rice cultivars. All the cultivars under study, except PR120 and PAU201, showed an increase in the amount of accumulation of 60 kDa polypeptide (proteins) with increase in N application.

Besides regular nutrition of plants for achieving high yields genotype, density and planting time play an important for good grain quality for bread making in wheat (Johansson *et al.* 2004). Optimum densities vary between areas, climatic conditions, planting time and varieties. Since cultivars genetically differ for yield components, individual cultivars need to be tested for proper density (Wiersma, 2002). Management practices play a vital role in determining yield and end-use quality of wheat. Many investigators have reported how N fertilization (Campillo *et al.* 2010; Hirzel *et al.* 2010; Nikolic *et al.* 2012), plant density, planting date affect yield and yield components of wheat (Otteson *et al.* 2008; Schillinger, 2005), which ultimately affects grain and bread making quality.

In wheat, grain yield and baking quality are dependent on the environment, genetic factors and the interaction between them (Yan and Holland 2010; Coventry *et al.* 2011). Similarly baking quality has been found to be influenced by sowing date (Singh *et al.* 2010). Flood, (1996) reported that bread quality was not badly affected by delayed sowing. In a field experiment, sowing date effects for bread quality were highly significant (Eslami, 2014). Bread quality score increased from first to last sowing date, with maximum score recorded at last sowing date. The interaction of variety and sowing date was also reported to be highly significant (Eslami, 2014). Adverse environmental condition during anthesis and grain filing are important factors in the baking quality classification of wheat (Jiang *et al.* 2009). Each sowing date determines the baking quality pattern of the wheat (Triboi- Blondel 2002; Motzo *et al.* 2007). An adequate sowing date positively impacts the grain yield (Silva *et al.* 2011) and baking quality of wheat, causing better adjustment to the phenology, physiology and environmental conditions (Ribeiro *et al.* 2009; Wheeler *et al.* 1996). However, it is difficult to obtain high grain and baking quality due to a negative association between these characteristics (Blanco *et al.* 2011).

Choosing the right sowing time can maximise the outcomes of interaction between genotype and environment, thus increasing grain yield and baking quality of wheat. In the work of Silva *et al.* (2011), wheat cultivars BRS Guamirim, BRS Guabiju and Safira showed high values for baking quality and differed significantly as affected by the baking quality characters.

Triticum aestivum species is considered to be rich source of the proteins glutenins and gliadin, and 21 discrete bands has been observed in the species (Galili, 1985). The number of bands observed depends on the environment and the type of method used to detect the protein. Along the same line, Hassan and Eid (1998) examined protein patterns of *Triticum aestivum*, *Tricticum durum*, and *Triticale* genotypes and found that glutenins, gliadins and subunits of these protein bands were varied in wheat. In addition Tarekegue *et al.* (2000) identified Ethopian wheat cultivars by grain protein electrophoresis on the basis of gliadins and glutenin subunit banding patterns, which were unique for all cultivars. In Egypt mature grains of two Egyptian and six imported wheat species (Triticum), were analysed for protein patterns using SDS-PAGE electrophoresis and total protein to characterise the variations between them. The results of the investigation revealed a total of 22 bands, which were distributed along the gel ranging from 6 to 143 KD. The differences in bands was attributed to the obvious variation in the number of position of bands from one species to another.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Experimental site

Field studies were conducted at the Botswana University of Agriculture and Natural Resources. Sebele, Gaborone, at $24^0$35'S, $25^0$56'E, and altitude 993 m, over the winter planting seasons of 2015 and 2016 under irrigation. Botswana is arid to semi-arid and winter temperatures recorded in the experimental site were minimum average of -2.3 °C during the month of July 2016 to a maximum average 37.2 °C in September. Total rainfall received was 49.5 mm (2015) and 35.8 mm (2016) for the crop growing months of April to September (Appendix 2).

3.2 Soil sampling and analysis

Before the crops were planted soil samples were taken from the field using zig- zag method (DAR, 1990) at a depth of 0.30 m using a soil auger. The soil samples were mixed to form a composite sample, then a representative was taken and analysed for physico-chemical properties; analysis of major elements, including nitrogen, phosphorus and potassium was performed (Table 3.1).

The following soil properties were determined: pH was done in a soil: water ratio of 1:2.5 in 0.01 $CaCl_2$; particle size with the hydrometer method; organic matter using Black and Walkey (1947) method; electrical conductivity (EC) in deionised water in a soil saturated paste; phosphorus in Bray II (1990) procedure; % N using Kjeldahl method and exchangeable cations (Ca, Mg, and K) after extraction with neutral ammonium acetate. All procedures are found in Page *et al.* (1982). The soil was acidic, suggesting that the basic cations have been leached out as confirmed by the measured data. Normally P is low in Botswana soils. However, because this site is an experimental field and there could have been a P build-up over the year (Refer to Table 3.2).

Table 3.1: Physico-chemical characteristics of the soil

Soil Property	2015	2016
pH	5.8	5.4
Organic carbon (%)	0.42	0.47
N (%)	0.0054	0.0043
P (mg/kg)	8.32	11.19
K (mol/kg)	0.23	0.37
Ca (mol/kg)	0.84	0.78
Mg (mol/kg)	0.26	0.32
Electrical conductivity (dSm^{-1})	0.39	0.24
Sand	68 %	
Silt	24 %	
Clay	18 %	
Textural class	Sandy-loam soil	

3.3 Experimental design and treatments

The experiment was laid out in a Randomised Complete Block Design (RCBD) under split-plot arrangement with three replications. The main plot consisted of two spring wheat genotypes, Baviaans and 14SAWYT308, and subplots comprised of two plant density treatments, being (0.2m × 0.15m) 333,333 plants/ha and (0.2m × 0.2m) 250 000 plants/ha; sub-sub plots were five nitrogen doses, being 0 kg/ha (N_0), 50 kg/ha (N_{50}), 75 kg/ha (N_{75}), 125 kg/ha (N_{125}) and 200 kg/ha (N_{200}); and sub-sub-sub plots of two planting dates being 21st April and 05th May. The nitrogen fertilizer applied was urea (46.5% N) and half of the nitrogen was applied at the time of planting while the remaining half was applied one week after seedling emergence. The experimental unit used was 1 meter by 3 meters with inter- row spacing of 0.2 meters. Two seeds per hole were sown manually at 0.15 m intra row spacing. Two weeks after sowing, thinning was done to one plant per hill to maintain a plant populations of 333,333 plants/ha and 250,000 plants/ha. Phosphorus (single super phosphate, SSP 9%) was applied at sowing at the rate of 55 kg/ha (DAR, 2012). Weeds were removed manually using hoes, and meteorological data were collected with assistance from Department of Agricultural Research meteorology station. Irrigation was done two times a week for two hours each time to field capacity, estimated according irrigation scheduling recommendations by Ngwako and Mashiqa (2013) from previous work.

3.4 Measurement of growth parameters and plant sampling

3.4.1 Growth

At 28, 56, 84, 112, and 140 days after sowing (DAS), three plants were randomly selected from each plot and measurements on leaf dry mass, leaf area duration, crop growth rate, net assimilation rate, and leaf area were taken. Regarding leaf dry mass the leaves from the three plants selected at random from every sub-plot were dried in the oven at 70 ± 5 C° until reaching a constant weight after 48 hours. The samples dry mass was used to compute leaf dry mass in g m^{-2}. For leaf area, leaves from each plant were placed on an electronic LI-3100 leaf area meter, USA- model and readings converted into square meters. Leaf area index (LAI) for each experimental unit was computed using leaf area values as the ratio of total leaf area to land for each experimental unit (Beadle, 1987).

3.4.1.1 Leaf area duration (LAD) (days)

Leaf area duration (LAD) was calculated by the formula as proposed by Hunt (1978). The LAD was determined starting from 28 DAS up to 112 DAS.

$$LAD = \frac{[(LAI_1 + LAI_2) \times (t_2 - t_1)]}{2}$$

LAI_1 = leaf area index at t_1

LAI_2 = leaf area index at t_2

T_1 = time for first observation

T_2 = time for second observation

3.4.1.2 Crop Growth Rate (CGR) (g $m^{-2}day^{-1}$)

Using the same plants as for LAD, dry matter accumulation was determined starting from 28 DAS up to 140 DAS by taking three plants at random from every sub-plot. After taking samples they were dried in the oven at 70 ± 5 C° until its constant weight. Then samples dry weight was calculated and used to find out crop growth rate by the formula as given by Hunt (1978).

$$CGR = \frac{W_2 - W_1}{t_2 - t_1} \ (gm^{-2}day^{-1})$$

W_2 = dry weight per unit land area (gm^{-2}) at second harvest

W_1 = dry weight per unit land area (gm^{-2}) at first harvest

T_2 = time taken to second harvest

T_1 = time to taken first harvest

3.4.1.3 Net Assimilation Rate (NAR) (g $m^{-2}day^{-1}$)

Net assimilation rate was determined by the formula as stated by Hunt (1978). The NAR was determined starting from 28 DAS up to 112 DAS using the same leaves as for CGR.

$$NAR = \frac{TDM}{LAD} \ (gm^{-2}day^{-1})$$

Where

TDM= Total dry matter

$$LAD = \frac{[(LAI_1 + LAI_2) \times (t_2 - t_1)]}{2}$$

LAI_1 = leaf area index at t_1

LAI_2 = leaf area index at t_2

T_1 = time for first observation

T_2 = time for second observation

3.4.2 Phenology

3.4.2.1Number of days to emergence

The date on which seedlings emerged was recorded for each experimental unit. Days to emergence was calculated as difference between date of emergence and date of sowing.

3.4.2.2 Number of days to anthesis

Date on which 75 percent of the spikes reached anthesis stage was recorded for each experimental unit. Days to anthesis was computed as difference between date of anthesis and date of sowing.

3.4.2.3 Number of days to physiological maturity

Date of physiological maturity was recorded for each experimental unit. Complete loss of green colour from glumes and peduncle was used as criteria for physiological maturity. Days to maturity was computed as difference between the date of physiological maturity and date of sowing.

3.4.3 Yield components

After physiological maturity, the following data were collected on ten randomly selected plants from each plots:

3.4.3.1 Spike length (cm)

Ten spikes were randomly selected from each plot and each spike measured using a ruler from base of the spike to the apex, and record the spike length in cm.

3.4.3.2 Number of spikelets per spike

From the ten spikes randomly selected from each plot, number of spikelets per spike were counted and an average taken and recorded as number of spikelets per spike.

3.4.3.3 Number of grains per spike

From the randomly selected ten spikes from each plot, each spike was threshed separately, and grain of each spike counted, and an average recorded.

3.4.3.4 1000- grain mass (g)

A sample of 1000- grains was taken at random from each plot and their weight taken using electric balance scale at constant mass.

3.4.3.5 Number of tillers per m^2

The number of productive tillers were counted from a sample of ten plants in each plot. An average was taken and the number computed to m^2.

3.4.4 Yield parameters

3.4.4.1 Biological Yield (kg/ha)

Thirty plants of each plot (two central rows) were harvested manually, and biological yield was recorded in kg by weighing the bundles of each plot using a digital laboratory balance (model 8800- Chicago, USA) scale, and the weight converted into kg/ha.

3.4.4.2 Grain Yield (kg/ha)

Wheat bundles of the two centre rows of each plot (1.5m^2) were sun dried to a moisture level of about 12% and threshed separately. This was achieved when the flag leaf and spikes turned yellow (Hanft and Wych, 1982). The grain mass of each experimental unit was weighed using an electric balance and recorded in kg and converted to kg/ha.

3.4.4.3 Straw yield (kg/ha)

Straw yield was calculated as biological yield value minus grain yield value.

3.4.4.4 Harvest Index (%)

Harvest index of each plot was calculated using formula:

$$HI = \frac{\text{Grain yield}}{\text{Biological yield}} * 100$$

3.4.5 Other parameters

3.4.5.1 Plant height at maturity (cm)

Ten plants randomly selected from each plot were measured for plant height using a ruler from the base of the node to the top, and recorded in cm.

3.4.5.2 Grain protein content (%)

Total content of nitrogen on grain samples was determined by micro Kjeldahl method described by Tecator (1991). Protein percentage was determined by multiplying grain nitrogen content by 5.7, which is the appropriate factor worked out for wheat (Winkleman *et al.*, 1990).

$$\text{Nitrogen (\%)} = \frac{\text{Volume of acid used} \times 0.0014 \times 250 \times 100}{\text{Sample weight} \times 10 \text{ ml}}$$

$$\text{Crude protein (\%)} = \text{\% Nitrogen} \times 5.7$$

3.5 Protein profiling methodology- Sodium Dodecyl Sulphate (SDS) Polyacrylamide Gel Electrophoresis method (SDS- PAGE).

Five wheat seeds from each of the 40 treatments were ground with an electric laboratory grinder (model A10 57- Kinematica Swirtzerland) and 10 mg of the seed flour was taken into a 1.5 mL micro tube. A 400 μL of protein extraction buffer (1 % β- mecaptoethanol, 5 M urea, 0.2% SDS, 0.05 M tris) and 0.0001 % bromo-phenol blue were added and content vortexed for 5 minutes. The crude homogenates were centrifuged using a micro centrifuge set at 13 000 rpm at room temperature for 10 minutes. The separation gel (12.25%) was prepared using 5 mL

Solution A, 7.5 ml Solution C, 200 µL of 10% Ammonium per sulphate (APS), 7.5 mL of distilled water and 15 µL of TEMED (Refer to Appendix 3). Glass plates were cleaned with 70% ethanol and fixed with clips, and separation gel poured to the cell layered with water, and after 30 minutes distilled water was removed and stacking gel added. A comb was inserted into stacking gel, and the supernatant was separated and 15 µL of each sample (supernatant) along with protein molecular weight marker (11 – 190 kDa broad range) was loaded into gels. Electrophosis was done at constant voltage of 100 volts, until the dye front reached the bottom of the gel. The gels were then removed from plates, dyed in staining solution (Coomasie Brilliant Blue – R250, 6% Acetic acid, 44% methanol) and shaken using an electric shaker (model MK 161- Japan) until protein bands appeared (2 hours). Gels were then washed with distilled water for 5 minutes then transferred to de-staining solution (20% methanol, and 5% acetic acid) then shaken in electronic shaker until protein bands appeared (2 hours). The gel were observed under gel documentation system Bio-RAD with white light illuminator and photographed.

3.6 Data analysis

Data on agronomic traits were subjected to a 4-way analysis of variance (ANOVA) using STATISTICA package, version 13.1 after testing for normality. Where there were significant differences, mean separation was done using the Least Significance Difference (LSD) method, and bar charts were constructed for significant interactions and physiological parameters (leaf dry mass, crop growth rate, leaf area duration, net assimilation rate and leaf area index) . Correlation was done for grain yield and yield parameters.

For protein profiling, data electrophoregrams for each treatment were scored and the presence (1) or absence (0) of each band recorded and entered into a binary data matrix, and similarity matrix generated was used to construct dendrogram using a statistical package NTSYC-PC, Version 2.0 (Rohlf, 2000).

CHAPTER FOUR

4.0 RESULTS

4.1 Effect of genotype, plant density, nitrogen rate, and planting date on wheat phenology

Table 4.1a shows the effect of genotypes, plant density, nitrogen application and planting date on the phenology of wheat. The genotype, plant density and the application of nitrogen fertiliser had no effect on the number of days to emergence of wheat. Planting date affected the number of days to emergence, with earlier planted wheat (21 April), emerging quicker (Table 4.1a). In 2016 similar results were observed as for 2015 (Table 4.1b).

With regards to anthesis, the period from the opening of the bud, wheat genotype and planting density had no effect on this parameter in 2015. Applying N fertiliser increased the number of days to anthesis. Plants that received 125 kg/ha of N exhibited the highest number of days to anthesis. Plants grown on 21st April reached the anthesis stage later than those planted on 5th May. In 2016, the wheat variety had no significant effect on the number of days to anthesis. Much like in the previous year, applying N fertiliser increased the number of days to anthesis with 125 kg/ha of N application rate exhibiting the highest number. Plants grown later (5th May) in the year reached anthesis quicker than those planted earlier (21st April).

In both years, regardless of whether Baviaans or 14SAWYT308 wheat genotypes were grown, the days to physiological maturity were not affected (Tables 4.1a and b). Furthermore, planting density and N application also did not influence the number of days to maturity of wheat. Interestingly, plants grown later in the year reached physiological maturity quicker than those planted earlier (Table 4.1b)

Table 4.1: (a) Effect of genotype, plant density, nitrogen rate, and planting date on number of days to emergence, number of days to anthesis and number of days to physiological maturity during 2015.

Days to emergence				
Genotype	**Plant density (plants/ha)**	**Nitrogen Rate (kg/ha)**		**Planting date**
Baviaans	333,333	0	6.14^{a}	21 April
6.33^{a}	6.63^{a}	50	6.29^{a}	6.08^{b}
		75	6.25^{a}	
14SAWYT308	250,000	125	6.67^{a}	5th May
6.52^{a}	6.23^{a}	200	6.50^{a}	6.77^{a}
Mean		6.43		
CV (%)		17.52		
LSD		0.41		
Days to anthesis				
Baviaans	333,333	0	84.7^{c}	21 April
86.33^{a}	86.43^{a}	50	86.40^{b}	88.23^{a}
		75	86.5^{b}	
14SAWYT308	250,000	125	87.45^{a}	5th May
86.42^{a}	86.32^{a}	200	86.8^{b}	84.52^{b}
Mean		86.38		
CV (%)		0.98		
LSD		0.31		
Days to physiological maturity				
Baviaans	333,333	0	143.25a	21 April
143.10^{a}	143.20^{a}	50	143.00a	146.00^{a}
		75	143.00a	
14SAWYT308	250,000	125	143.00a	5th May
143.10^{a}	143.00^{a}	200	143.25a	140.20^{b}
Mean		143.10		
CV (%)		0.54		
LSD		0.28		

Means in a column with same letters are statistically not significant at $p \leq 0.05$.

Table 4.1:(b) Effect of genotype, plant density, nitrogen rate, and planting date on number of days to emergence, number of days to anthesis and number of days to physiological maturity during 2016.

Genotype	**Plant density (plants/ha)**	**Nitrogen rate (kg/ha)**		**Planting date**
		Days to emergence		
Baviaans	333,333	0	6.17^{a}	**21 April**
5.98^{a}	6.33^{a}	50	5.92^{a}	5.93^{b}
		75	6.08^{a}	
14SAWYT308	250,000	125	6.25^{a}	5th May
6.35^{a}	6.00^{a}	200	6.42^{a}	6.40^{a}
Mean		6.17		
CV (%)		17.67		
LSD		0.39		
		Days to anthesis		
Baviaans	333,333	0	83.71^{ab}	**21 April**
84.52^{a}	86.23^{b}	50	84.58^{ab}	85.48^{a}
		75	84.54^{ab}	
14SAWYT308	250,000	125	85.17^{a}	5th May
84.63^{a}	84.95^{a}	200	84.88^{a}	83.67^{b}
Mean		84.58		
CV (%)		2.04		
LSD		0.63		
		Days to physiological maturity		
Baviaans	333,333	0	144.00^{a}	**21 April**
144.10^{a}	144.10^{a}	50	144.33^{a}	147.97^{a}
		75	144.00^{a}	
14SAWYT308	250,000	125	144.00^{a}	5th May
144.10^{a}	144.00^{a}	200	143.92^{a}	140.13^{b}
Mean		144.10		
CV (%)		0.52		
LSD		0.27		

Means in a column with same letters are statistically not significant at $p \leq 0.05$.

4.2 Effect of genotype, plant density, nitrogen rate and planting date on yield related parameters of wheat genotypes

In 2015 there were significant differences between genotypes ($p \leq 0.001$) in number of tillers per square meter, spike length, number of grains per spike, 1000- grain mass, plant height but not in the number of spikelets per spike (Table 4.2a). The genotype 14SAWYT308 had significantly higher number of tillers per m^2, number of grains per spike, 1000 grain mass and plant height compared to Baviaans. Baviaans had a higher spike length compared to 14SAWYT308.

All the yield components under study and plant height were significantly influenced by plant density, during both years (Table 4.2a and b). Significantly higher mean values were obtained from the 333,333 plants/ha treatment. In both years, application of N significantly influenced the yield components of wheat. The 125 kg/ha N application rate exhibited the highest number of tillers/ m^2, spike length, number of spikelets/spike, 1000 grain mass, and plant height. Furthermore, planting date had a significant effect on the yield components of wheat. The earlier planted wheat plants had higher number of tillers/ m^2, spike length, number of spikelets/spike, number of grains/spike and taller plants. In 2015 there were no significant differences in the 1000 grain mass between the two planting densities.

In 2016, genotype 14SAWYT308 gave significantly longer spikes and more number of spikelets per spike than Baviaans (Table 4.2b). In the same year plant density of 333,333 plants/ha produced more 1000-grain mass, more number of tillers, spike length, number of spikelets per spike, number of grains per spike and plant height. Nitrogen and early planting gave more number of tillers, number of spikelets per spike, number of grains per spike, 100-grain mass and plant height.

Table 4.2: (a) Effect of genotype, planting density, nitrogen and planting date on yield related parameters of wheat genotypes during 2015 season

	number of tillers/m^2	**spike length(cm)**	**number of spikelets per spike**	**number of grains per spike**	**1000 grain mass(g)**	**Plant height(cm)**
Treatments						
Genotype						
Baviaans	320.5±9.7b	10.8±0.1a	17.3±0.2a	48.8±1.0b	39.5±0.5b	76.6±1.3b
14SWAYT308	335.5±9.3a	10.4±0.2b	17.2±0.3a	50.9±0.9a	40.1±0.5a	79.2±1.4a
Planting density						
333,333 plants/ha	373.9±9.1a	10.8±0.2a	17.8±0.2a	52.4±1.1a	39.9±0.5a	79.4±1.4a
250,000 plants/ha	282.1±5.4b	10.4±0.2b	16.6±0.2b	47.3±0.7b	39.6±0.5a	76.5±1.3b
Nitrogen(N) rate						
0	274.5±12.0e	9.5±0.3d	15.5±0.4d	48.3±0.9cd	37.0±0.5d	66.5±0.6e
50	306.3±5.9c	10.8±0.2b	17.8±0.3bc	49.7±1.3bc	39.8±0.5c	70.8±0.7d
75	368.9±13.0b	10.1±0.2c	17.3±0.3c	47.1±1.5d	42.6±0.7b	75.2±0.5c
125	397.3±16.5a	11.3±0.3a	18.5±0.2a	54.1±2.0a	43.3±0.5a	94.8±0.8a
200	295.5±9.7d	11.3±0.3a	17.0±0.4cd	49.9±1.5b	36.3±0.4d	82.6±0.6b
Planting date						
21April	347.3±9.5a	11.1±0.2a	17.5±0.3a	51.6±1.2a	40.4±0.5a	78.7±1.4a
05 May	308.7±8.9b	10.2±0.1b	16.9±0.2b	48.1±0.7b	39.2±0.5b	77.2±1.3b
F statistics						
Genotype	99.2***	9.87**	0.00ns	20.50***	5.18*	37.1***
Planting density	3645.2***	13.92***	56.03***	110.96***	1.96ns	44.5***
N rate	922.2***	35.85***	36.23***	25.15***	128.69***	575.3***
Planting date	643.0***	59.04***	10.77*	47.56***	22.45***	14.0***
Genotype*density*N rate* planting date	9.6***	3.59**	4.61**	12.46***	3.66**	0.5ns
CV %	2.53	5.94	5.13	5.30	3.33	2.59
LSD	3.02	0.23	0.32	0.96	0.48	0.73

Means ±SE in a column with same letters are statistically not significant at $p\leq0.05$ (*); $p\leq0.01$ (**); $p\leq 0.001$(***).

Table 4.2: (b) Effect of genotype, planting density, nitrogen and planting date on yield related parameters of wheat genotypes during 2016 season

	number of tillers/m²	spike length(cm)	number of spikelets per spike	number of grains per spike	1000 grain mass(g)	Plant height(cm)
Treatments						
Genotype						
Baviaans	323.1±9.8b	10.6±0.1b	16.6±0.1b	44.9±0.7b	39.5±0.5b	76.3±1.5b
14SWAYT308	337.5±9.4a	10.8±0.2a	17.3±0.2a	47.6±0.8a	40.8±0.5a	79.2±1.4a
Planting density						
333,333 plants/ha	375.4±9.5a	11.0±0.2a	17.4±0.2a	48.3±0.9a	40.6±0.5a	79.0±1.6a
250,000 plants/ha	285.2±5.2b	10.4±0.1b	16.5±0.2b	44.2±0.5b	39.7±0.5b	76.5±1.3b
Nitrogen(N) rate						
0	274.9±10.9d	9.8±0.1c	14.8±0.2d	44.0±0.5c	36.7±0.5c	62.3±0.7e
50	301.0±5.2c	10.9±0.2a	17.2±0.2bc	44.8±0.7c	40.4±0.5b	72.5±0.6d
75	367.1±12.7b	10.6±0.2b	17.1±0.3c	43.1±1.0d	43.0±0.5a	77.4±0.4c
125	407.3±16.8a	11.0±0.2a	18.2±0.2a	51.1±1.6a	43.6±0.6a	94.2±1.1a
200	303.8±9.9c	11.0±0.3a	17.4±0.3b	48.2±1.2b	37.2±0.4c	82.7±0.7b
Planting date						
21April	347.5±9.8a	11.3±0.2a	17.3±0.2a	47.5±0.9a	41.3±0.4a	78.8±1.6a
05 May	313.0±9.0b	10.1±0.1b	16.6±0.2b	45.0±0.5b	39.0±0.5b	76.7±1.3b
F statistics						
Genotype	218.9***	12.9***	56.4***	110.2***	25.3***	200.0***
Planting density	8446.4***	132.5***	95.6***	258.9	12.2***	144.7***
N rate	2437.2***	64.8***	172.8***	131.3***	128.3***	2731.3***
Planting date	1216.3***	477.1***	67.4***	77.4***	83.6***	117.1***
Genotype*density*N rate* planting date	17.8***	28.3***	0.3ns	14.8***	2.9*	6.2***
CV %	1.62	2.81	2.55	2.67	3.28	1.21
LSD	1.94	0.11	0.16	0.45	0.47	0.34

Means ±SE in a column with same letters are statistically not significant at $p \leq 0.05$ (*); $p \leq 0.01$ (**); $p \leq 0.001$(***).

4.3 Effect of genotype, plant density, nitrogen rate and planting date on yield parameters of wheat genotypes

There were significant differences in straw, grain, biological yields, and harvest index between the two genotype during the 2015 season (Table 4.3a). In all of these parameters, 14SAWYT308 exhibited higher mean values compared to Baviaans.

In 2015 planting density had no significant effect on the wheat straw yield. However, the grain and biological yield were significantly higher for crops planted at 333,333 plants/ha. Therefore, logically the harvest index was significantly higher for the higher density.

With respect to the effect of nitrogen application on the yield parameters, there were significant differences among the application rates of N for all parameters. The 125 kg/ha of N exhibited the highest straw, grain, and biological yields as well as the highest harvest index. Plants grown on 21st April had higher yield parameters compared to those planted on the 5th May (Table 4.3a).

In 2016, a similar trend was found for all the treatments as in 2015 with 14SAWYT308 wheat genotype, the 333,333 planting density, the 125 kg/ha of N application rate and the earlier planting density exhibiting the highest yield parameters i.e. straw yield, grain yield, biological yield and harvest index (Table 4.3b).

Table 4.3: (a) Effect of genotype, planting density, nitrogen and planting date on yield parameters of wheat genotypes during 2015 season

	Straw Yield (kg/ha)	**Grain Yield (kg/ha)**	**Biological yield (kg/ha)**	**Harvest Index (%)**
Treatments				
Genotype				
Baviaans	4267.2 ±116.8b	2562.4 ±130.0b	6829.6 ±238.0b	36.6 ±0.8b
14SWAYT308	4533.1 ±115.7a	2859.9 ±137.6a	7393.0 ±247.6a	37.7 ±0.7a
Planting density				
333,333 plants/ha	4397.9 ±120.9a	2871.8 ±142.1a	7269.8 ±255.0a	38.6 ±0.7a
250,000 plants/ha	4402.3 ±114.0a	2550.5 ±124.6b	6952.8 ±234.0b	35.7 ±0.7b
Nitrogen (N) rate				
0	3570.7 ±104.1e	1467.9 ±42.6e	5038.6 ±117.9e	29.5 ±1.2e
50	3747.8 ±71.5d	2266.3 ±78.8d	6014.1 ±135.9d	37.5 ±0.7c
75	4812.1 ±115.6b	3155.0 ±94.4b	7967.1 ±200.0b	39.5 ±0.4b
125	5468.2 ±172.0a	4083.0 ±195.6a	9551.2 ±355.1a	42.4 ±0.7a
200	4446.2 ±113.6c	2620.7 ±114.6c	7066.9 ±215.0c	36.8 ±0.6d
Planting date				
21 April	4680.0 ±141.1a	3073.5 ±152.7a	7753.5±285.7a	38.7±0.7a
05 May	4120.3 ±71.2b	2348.8 ±93.9b	6469.1±158.1b	35.5 ±0.7b
F statistics				
Genotype	23.18***	105.25***	58.82***	7.66*
Planting density	0.23ns	123.43***	17.01***	53.12***
N rate	188.87***	983.09***	509.36***	113.56***
Planting date	128.98***	688.86***	357.80***	62.15***
Genotype*density*N rate* planting date	1.84ns	7.89***	0.08ns	7.99***
CV %	5.06	3.88	3.61	5.95
LSD	80.85	38.21	93.17	0.80

Means ±SE in a column with same letters are statistically not significant at $p \leq 0.05$ (*); $p \leq 0.01$ (**); $p \leq 0.001$(***).

Table 4.3: (b) Effect of genotype, planting density, nitrogen and planting date on yield parameters of wheat genotypes during 2016 season

	Straw Yield (kg/ha)	Grain Yield (kg/ha)	Biological yield (kg/ha)	Harvest Index (%)
Treatments				
Genotype				
Baviaans	4211.1±106.1b	2586.9±134.8b	6798.0±236.1b	36.8±0.7b
14SWAYT308	4544.9±114.5a	2894.1±142.5a	7439.0±254.6a	37.8±0.6a
Planting density				
333,333 plants/ha	4533.0±116.9a	2940.6±148.7a	7473.6±262.5a	38.2±0.7a
250,000 plants/ha	4223.1±104.1b	2540.3±125.7b	6763.4±225.4b	36.4±0.7b
Nitrogen (N) rate				
0	3451.5±62.9e	1416.5±45.6e	4868.0±94.7e	29.0±0.6e
50	3710.4±77.5d	2311.9±71.6d	6022.3±142.9d	38.3±0.4c
75	4793.8±84.4b	3170.9±96.2b	7964.6±174.9b	39.7±0.4b
125	5446.3±142.8a	4174.1±199.3a	9620.4±212.2a	42.9±0.6a
200	4533.3±98.1c	2664.7±119.0c	7198.0±212.2c	36.7±0.6d
Planting date				
21 April	4630.4±127.8a	3094.9±158.4a	7725.3±282.4a	38.8±0.7a
05 May	4125.6±82.7b	2386.1±99.6b	6511.7±178.2b	35.8±0.6b
F statistics				
Genotype	71.61***	174.54***	134.15***	23.0***
Planting density	61.25***	299.92***	165.90***	71.5***
N rate	376.60***	1634.58***	936.81***	506.3***
Planting date	192.17***	999.86***	539.00***	218.7***
Genotype*density*N rate* planting date	1.43ns	11.46***	0.64ns	17.9***
CV %	3.48	3.25	2.62	3.03
LSD	55.43	32.35	67.64	0.41

Means ±SE in a column with same letters are statistically not significant at p≤0.05 (*); p≤0.01 (**); p≤ 0.001(***).

4.4 Mean comparisons of genotype, plant density, nitrogen rate and planting date on grain protein content of wheat genotypes in 2015 and 2016

There were significant differences in the percentage grain protein for the various treatments in 2015 and 2016 (Table 4.4). The 14SAWYT308 exhibited higher protein content in both years compared to Baviaans. Planting density did not affect the protein content of wheat. N application at the rate of 125 kg/ha gave significantly higher protein content than all the other N rates. The early planted crops led to higher grain protein content in both years.

Table 4.4 Effect of genotype, plant density, nitrogen rate and planting date on grain protein content of wheat genotypes during 2015 and 2016 seasons

Grain Protein Content (%) of wheat genotypes

	2015	2016
Treatments		
Genotype		
Baviaans	12.3 ±0.1b	12.7±0.1b
14SAWYT308	12.8 ±0.2a	13.1±0.1a
Planting density		
333,333 plants/ha	12.6 ±0.2a	12.9±0.1a
250,000 plants/ha	12.5 ±0.1a	12.9±0.1a
Nitrogen (N) rate		
0	11.2 ±0.1d	11.8±0.1e
50	12.5 ±0.1c	13.0±0.1c
75	13.2 ±0.1b	13.4±0.1b
125	13.6 ±0.4a	13.7±0.2a
200	12.3 ±0.1c	12.7±0.1d
Planting date		
21 April	12.8±0.2a	13.1±0.1a
05 May	12.3 ±0.1b	12.7±0.1b
F statistics		
Genotype	21.73***	54.9***
Planting density	0.56ns	0.6ns
N rate	53.78***	114.4***
Planting date	21.38***	51.7***
Genotype*density*N rate* planting date	2.20ns	4.9**
CV %	4.91	2.58
LSD	0.22	0.12

Means ±SE in a column with same letters are statistically not significant at $p \leq 0.05$ (*); $p \leq 0.01$ (**); $p \leq 0.001$(***).

4.5 Effect of genotype on leaf area of wheat genotypes

Genotype significantly enhanced the leaf area of both genotypes at all stages of growth, except at early stage (Figure 4.1). Leaf area increase gradually from vegetative stage (28 DAS) until it declined as it reached early maturity stage (112 DAS). A similar trend occurred in 2016 as shown in Figure 4.2.

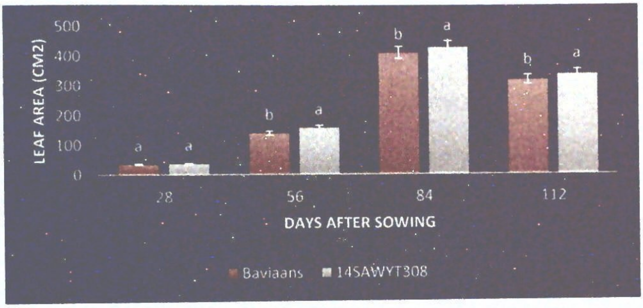

Figure 4.1: Effect of genotype on leaf area of wheat during 2015.

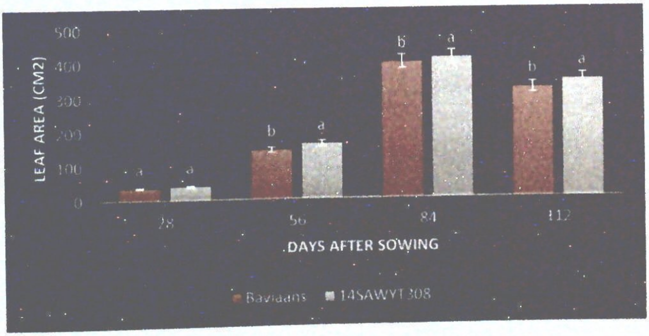

Figure 4.2: Effect of genotype on leaf area of wheat during 2016.

4.6 Effect of planting density on physiological traits of wheat

Figure 4.3 shows the effect of plant density on leaf dry mass of wheat genotype during the 2015 season. From vegetative stage (28 DAS) leaf dry matter accumulation for density of 333,333 plants/ha did not differ significantly to that of 250,000 plants/ha. The leaf dry matter values increased from head development stage (56 DAS) until they reached maximum values at flowering stage (84 DAS), and thereafter dropped until plants reached maturity. A similar pattern was observed for the 2016 season (Figure 4.4), where maximum leaf dry mass was recorded at flowering stage for 333,333 plants/ha, but did not differ significantly compared to the 250,000 plants/ha density at flowering.

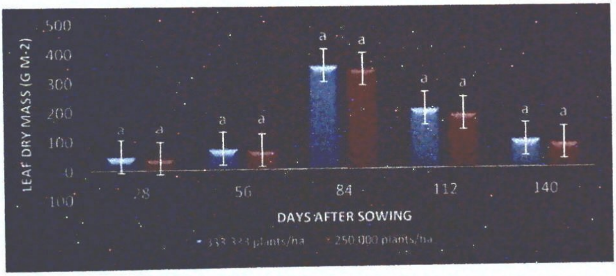

Figure 4.3: Effect of plant density on leaf dry mass (g m^{-2}) of wheat at 28 to 140 DAS during 2015

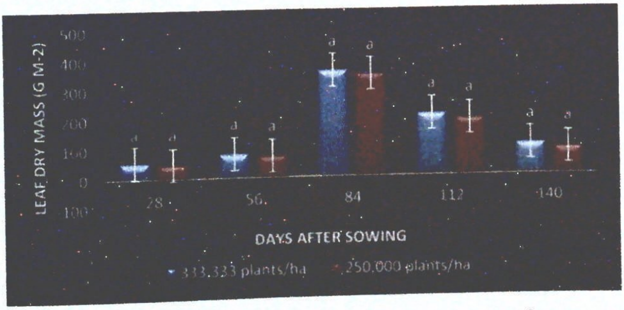

Figure 4.4: Effect of plant density on leaf dry mass (g m^{-2}) of wheat from 28 to 140 DAS during 2016.

A similar trend was observed for crop growth rate and leaf area index where the peak values were observed at flowering stage in both years (Figure 4.5 and 4.6).

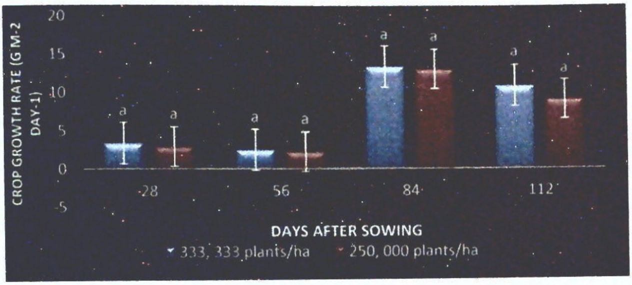

Figure 4.5: Effect of plant density on crop growth rate (g m^{-2} day^{-1}) in wheat genotypes from 28 to 112 DAS during 2015

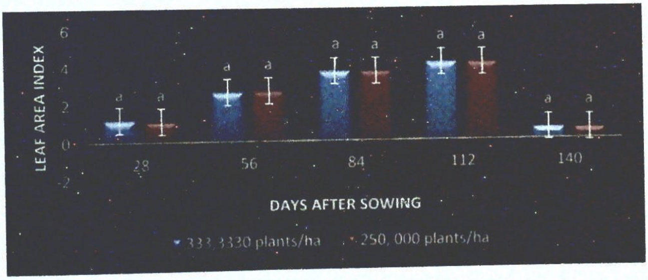

Figure 4.6: Effect of plant density on leaf area index of wheat genotypes from 28 to 140 DAS during 2015.

Regarding net assimilation rate (NAR), from the vegetative stage (28 DAS), the values dropped sharply until head development stage (56 DAS), thereafter increased and reached maximum at flowering stage (84 DAS) (Figure 4.7). However, the net assimilation rate was not significantly affected by density during all the sampling dates. A similar pattern occurred during 2016, where plant density had no significant effect on net assimilation rate in all sampling stages of plant growth (Figure 4.8).

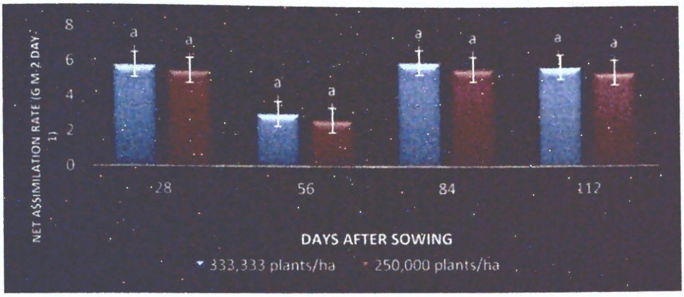

Figure 4.7: Effect of plant density on net assimilation rate (g m^{-2} day^{-1}) in wheat genotypes from 28 to 112 DAS during 2015.

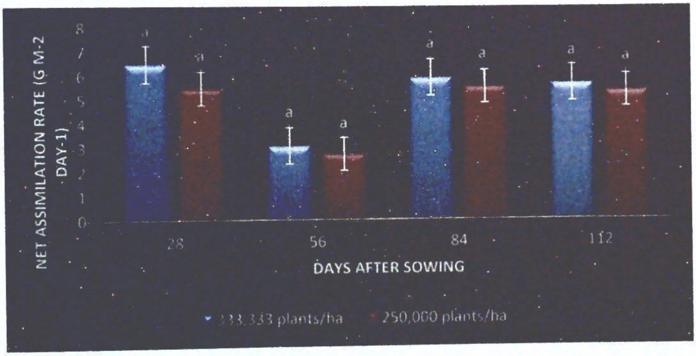

Figure 4.8: Effect of plant density on net assimilation rate (g m^{-2} day^{-1}) in wheat genotypes from 28 to 112 DAS during 2016.

4.7 Effect of nitrogen application rate on some physiological traits of wheat

Nitrogen enhanced leaf dry matter of wheat genotypes in both years. During 2015 at vegetative stage (28 DAS), more leaf dry mass was accumulated with 125 kg/ha of N, followed by N_{75}, N_{50} and N_{200} (Figure 4.9) but the means did not differ significantly from each other ($p \leq 0.05$) for a particular sampling date. Leaf dry mass accumulation increased steadily from the vegetative stage until it reached peak at flowering stage (84 DAS), thereafter reduced gradually until physiological maturity stage (140 DAS). During the 2016 season, a similar trend occurred where maximum leaf dry was recorded at 125 kg/ha of N at flowering stage, thereafter declining steadily until physiological maturity period (Figure 4.10).

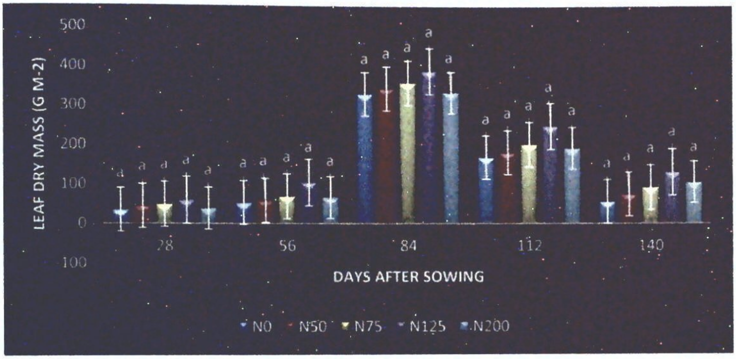

Figure 4.9 Effect of nitrogen doses on leaf dry mass (g m^{-2}) in wheat at 28 to 140 DAS during 2015.

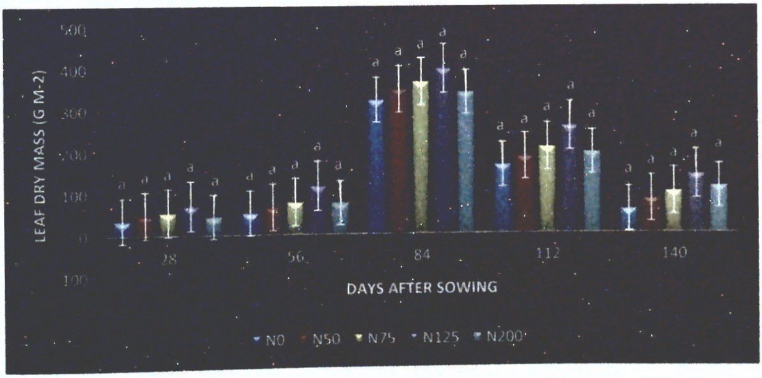

Figure 4.10: Effect of nitrogen doses on leaf dry mass (g m^{-2}) in wheat from 28 to 140 DAS during 2016.

Crop growth rate and leaf area index (LAI) followed a similar trend to leaf dry mass, where the highest effects were observed at flowering in both years (Figure 4.11 and 4.12).

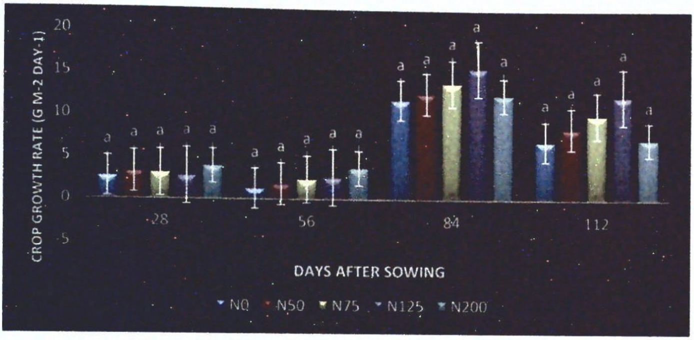

Figure 4.11: Effect of nitrogen on crop growth rate (g m^{-2} day^{-1}) in wheat from 28 to 112 DAS during 2015.

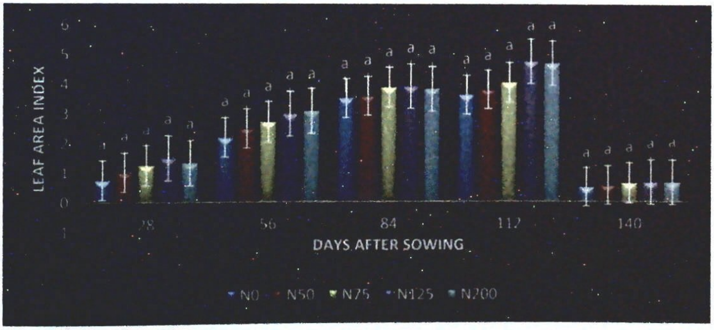

Figure 4.12: Effect of nitrogen on leaf area index in wheat from 28 to 140 DAS during 2015.

During 2015 the NAR decreased from vegetative stage (28 DAS) up to head development stage (56 DAS) then increased steadily until it reached a maximum value at flowering stage (84 DAS), then decreased maturity stage (Figure 4.13). Similarly in 2016, maximum net assimilation rate was recorded at 125 kg/ha of N at flowering stage, while the minimum net assimilation rate was obtained from nitrogen dose of 200 kg/ha, 56 days after planting (Figure 4.14).

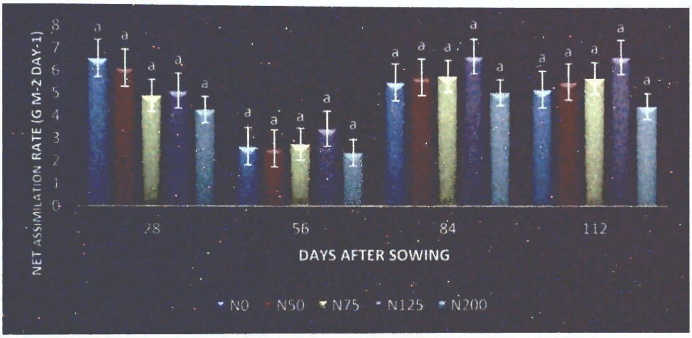

Figure 4.13: Effect of nitrogen on net assimilation rate (g m^{-2} day^{-1}) in wheat from 28 to 112 DAS during 2015.

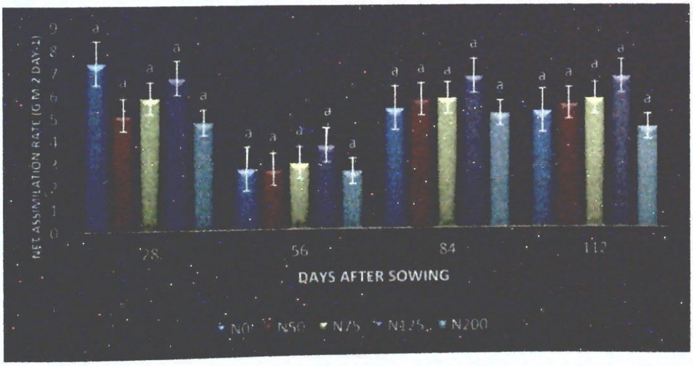

Figure 4.14: Effect of nitrogen on net assimilation rate (g m^{-2} day^{-1}) in wheat from 28 to 112 DAS during 2016.

4.8 Effect of planting date on some physiological traits of wheat

The effect of planting date on leaf dry mass of wheat during 2015 is depicted in Figure 4.15. During early days of growth (28 DAS) accumulation of leaf dry mass was lower for both dates, and increased at head development stage (56 DAS). Thereafter, values quickly rose until peak at flowering stage (84 DAS), before sharply decreasing until physiological maturity. The highest leaf dry mass recorded at 84 DAS. However, there were no differences between the two planting dates on each sampling occasion. A similar pattern was recorded during the 2016 season where maximum leaf dry mass was observed for the early planting treatment at flowering compared to a slightly lower value for later planting date (Figure 4.16).

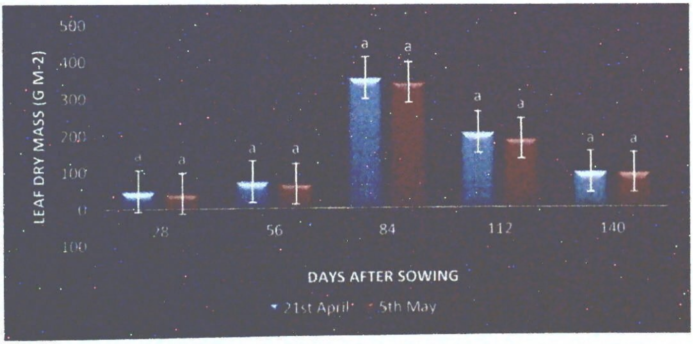

Figure 4.15: Effect of planting date on leaf dry mass (g m^{-2}) in wheat from 28 to 140 DAS during 2015.

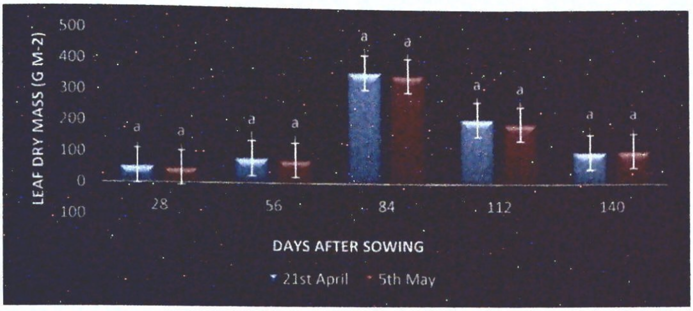

Figure 4.16: Effect of planting date on leaf dry mass (g m^{-2}) in wheat from 28 to 140 DAS during 2016.

Crop growth rate decreased slightly from early vegetative growth (28 DAS) up to 56 DAS, where it increased sharply to reach maximum at flowering stage, thereafter dropped until crops reached maturity. Highest crop growth was recorded on 21st April date, at flowering stage (84 DAS) during the 2015 planting, compared to a slightly lower crop growth rate value of later planting at the same 84 DAS period (Figure 4.17). Leaf area index during both years followed a similar pattern to that of leaf dry mass where higher values were reached at flowering, then decline steadily until maturity in both years (Figures 4.18).

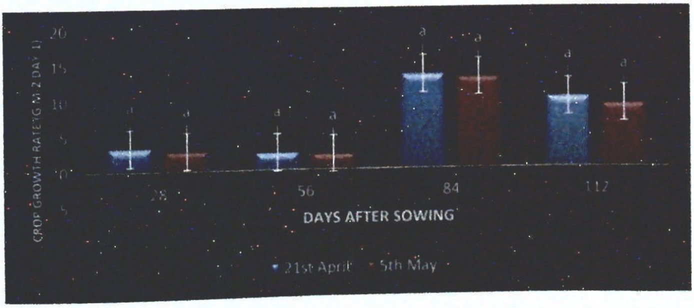

Figure 4.17: Effect of planting date on crop growth rate (g m^{-2} day^{-1}) in wheat from 28 to 112 DAS during 2015.

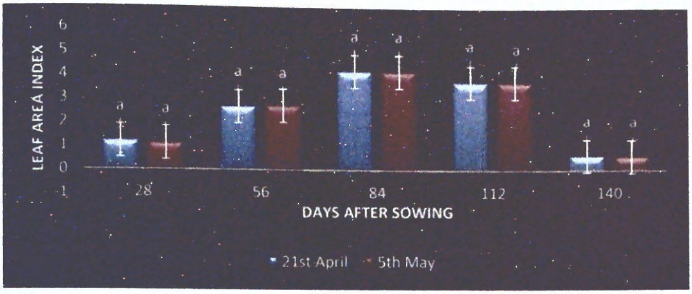

Figure 4.18: Effect of planting date on leaf area index in wheat from 28 to 140 DAS during 2015.

Responses of wheat to planting date on NAR during 2015 and 2016 are shown in Figures 4.19 and 4.20. The NAR was high in the initial growing process, then declined at head development stage (56 DAS), then increased rapidly up to flowering stage, where it reached peak, thereafter declined slowly until maturity stage.

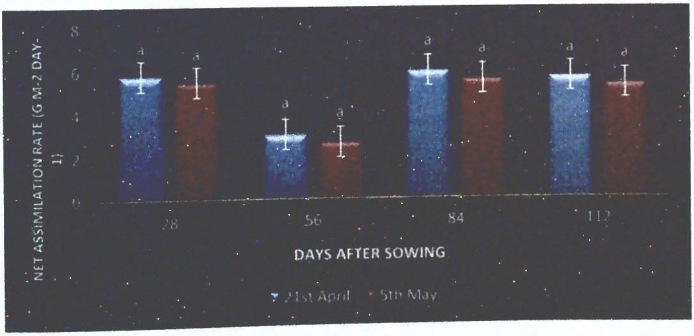

Figure 4.19: Effect of planting date on net assimilation rate (g m^{-2} day^{-1}) in wheat from 28 to 112 DAS during 2015.

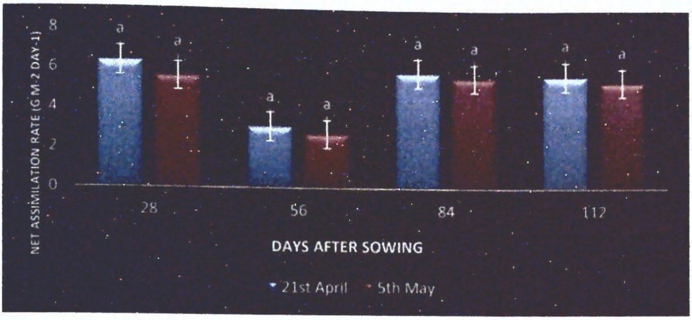

Figure 4.20: Effect of planting date on net assimilation rate (g m^{-2} day^{-1}) in wheat from 28 to 112 DAS during 2016.

4.9 Relationship between grain yield and yield components

The relationship between grain yield and yield components of wheat is shown in Table 4.5. Correlation analysis shows a positive significant relationship between grain yield and other traits, at ($p \leq 0.05$). The highest significant correlation was between grain yield and number of tillers (r=0.72), while the lowest correlation was between spike length and 1000 grain mass, and was non-significant (r=0.12).

Table 4.5: Correlation coefficient among grain yield and yield components of wheat

Plant characters	Number of tillers/m^2	Spike length(cm)	Number of spikelets/spike	Number of grains/spike	1000-grain mass(g)
Number of tillers/m^2	1.00				
Spike length(cm)	0.38*	1.00			
Number of spikelets/spike	0.57*	0.52*	1.00		
Number of grains/spike	0.49*	0.52*	0.36*	1.00	
1000- grain mass(g)	0.55*	0.12ns	0.36*	0.21*	1.00
Grain yield (kg/ha)	0.72*	0.39*	0.51*	0.41*	0.63*

4.10. Effect of plant density and planting date interaction on wheat

4.10.1. Effect of plant density and planting date on number of tillers per square meter

There was a significant interaction between planting density and planting date on the number of wheat tillers in 2015. For both planting dates, there were significantly more tillers for the higher density compared to the lower one. Furthermore, wheat planted on the 21 April, produced significantly more tillers relative to those planted on the 05th May date (Figure 4.21). A similar trend was observed in 2016 (Figure 4.22).

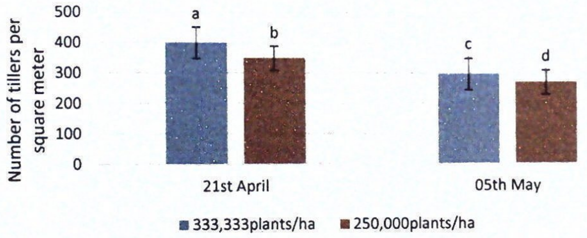

Figure 4.21: Interactive effects of plant density by planting date on number of tillers per square meter during 2015

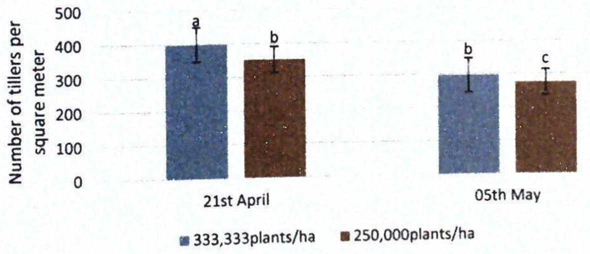

Figure 4.22: Interactive effects of plant density by planting date on number of tillers per square meter during 2016

4.10.2 Effect of plant density and planting date on number of spikelets per spike

In 2015, a significant interactive effect of plant density and planting date was revealed for the number of spikelets per spike as reflected in Figure 4.23. With the 21st April planting date, a significantly higher number of spikelets per spike was produced under 333,333 plants/ha density, compared to that of 250,000 plants/ha under same 21st April planting date. However, the interaction of plant density and planting date did not significantly influence number of spikelets per spike for the 05th May planting date. A similar pattern occurred in 2016 where plant density and planting date interaction significantly influenced number of spikelets per spike (Figure 4.24).

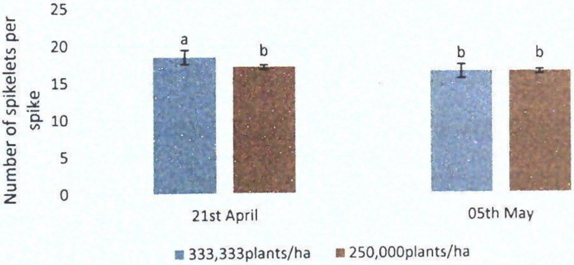

Figure 4.23: Interactive effects of plant density by planting date on number of spikelets per spike during 2015

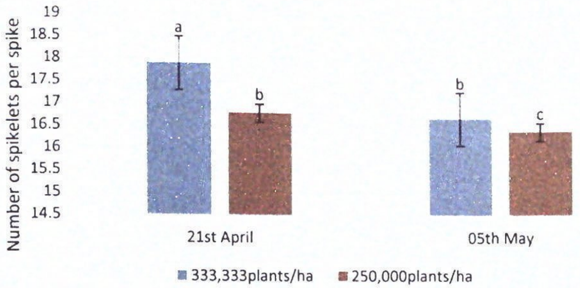

Figure 4.24: Interactive effects of plant density by planting date on number of spikelets per spike during 2016

4.10.3 Effect of plant density and planting date on number of grains per spike of wheat

The interaction of plant density and planting date was significant for number of grains per spike during 2015 (Figure 4.25).With reference to 21st April planting date, a higher grain number per spike was produced from 333,333 plants/ha density, significantly different from the 250,000 plants/ha density. In comparison, the 05th May planting date produced lower number of grains per spike, with maximum number of grains per spike obtained from 250,000 plants/ha density, significantly differing from that of 333,333 plants/ha density.

More grains per spike were produced on 21st April planting than 05th May planting date in 2016. Significantly higher number of grains per spike was recorded at 333,333 plants/ha, during the 21st April planting, but for 05th May planting date plant density had no significant impact on number of grains per spike of wheat (Figure 4.26).

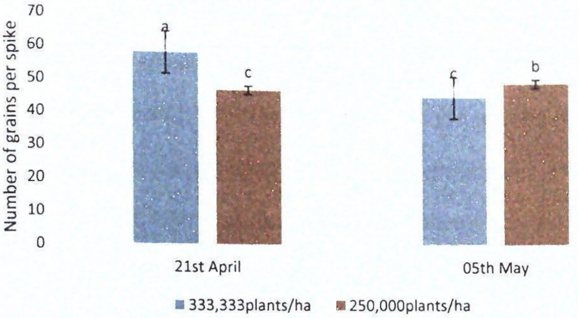

Figure 4.25: Interactive effects of plant density by planting date on number of grains per spike during 2015

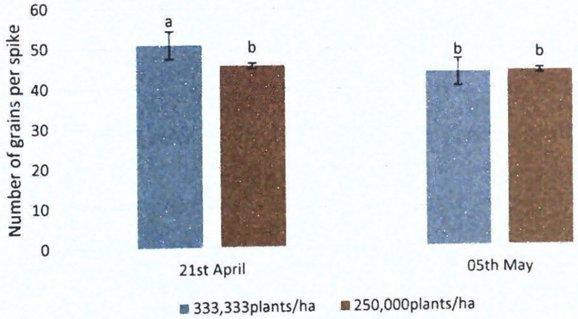

Figure 4.26: Interactive effects of plant density by planting date on number of grains per spike during 2016

4.10.4 Effect of plant density and planting date on biological yield of wheat

Plant density and planting date significantly interacted and influenced the biological yield of wheat during the 2015 season (Figure 4.27). The biological yield produced from 333,333 plants/ha treatment was significantly higher than that of the 250,000 plants/ha density. A similar trend was apparent for the 05th May planting time. However, biological yield obtained from the higher density was lower than during the first planting time. Likewise in 2016 the 21st April planting with 333,333 plants/ha exhibited significantly higher biomass than the 21st April planting with 250,000 plants/ha interaction. Similarly the 05th May planting with 333,333 plants/ha also showed the same trend of significantly higher biological yield than the 05th May treatment with 250,000 plants/ha interaction. (Figure 4.28).

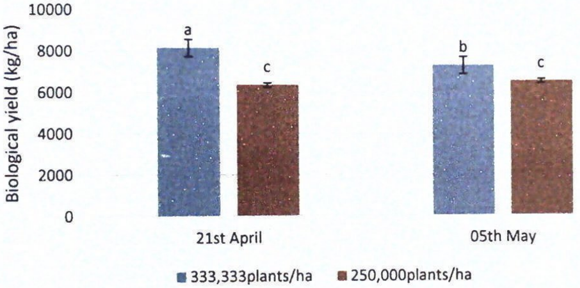

Figure 4.27: Interactive effects of plant density by planting date on biological yield during 2015

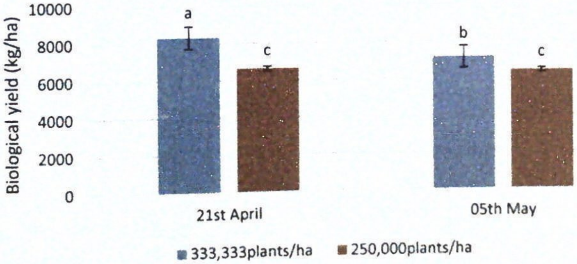

Figure 4.28: Interactive effects of plant density by planting date on biological yield during 2016

4.10.5 Effect of plant density and planting date on grain yield of wheat

Figure 4.29 shows the effect of planting date and plant density on grain yield of wheat during 2015. Significant grain yield value was recorded at 333,333 plants/ha during 21st April planting. The 05th May planting at 333,333 plants/ha produced significantly higher grain yield compared to 250,000 plants/ha.

A similar pattern was observed during 2016 season where 333,333 plants/ha recorded more grain yield than 250,000 plants/ha for both planting dates (Figure 4.30).

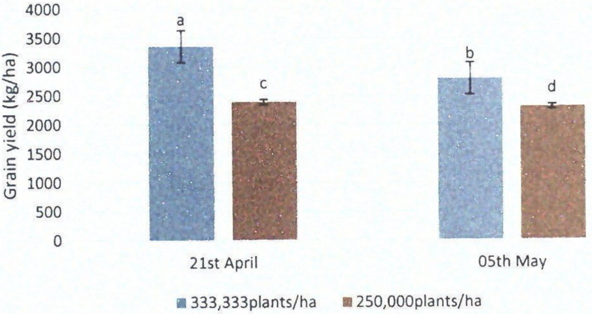

Figure 4.29: Interactive effects of plant density by planting date on grain yield during 2015

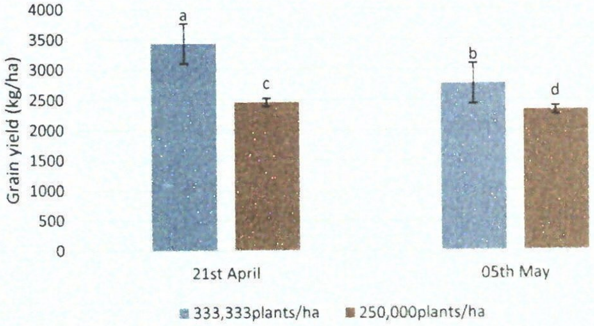

Figure 4.30: Interactive effects of plant density by planting date on grain yield during 2016

4.11 Effect of plant density and nitrogen application rate interaction on wheat

4.11.1 Effect of plant density and nitrogen application rate on the number of tillers

A significant interactive effect of plant density and nitrogen application rate on the number of tillers of wheat was observed during the 2015 season (Figure 4.31). Under the 333,333 plants/ha density, a higher number of tillers was obtained at nitrogen application rate of 125 kg/ha, and was significantly different from those at 250,000 plants/ha density at the same 125 kg/ha nitrogen rate. Increased plant density significantly enhanced the number of tillers in all nitrogen treatments except for the 50 kg/ha of N.

Similarly in 2016, the highest number of tillers was produced from 333,333 plants/ha treatment relative to 250,000 plants/ha, with higher values at 125 kg/ha of N (Figure 4.32).

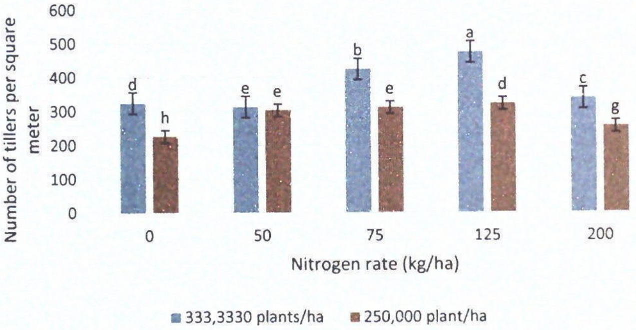

Figure 4.31: Interactive effect of plant density and nitrogen application rate on number of tillers per square meter during 2015

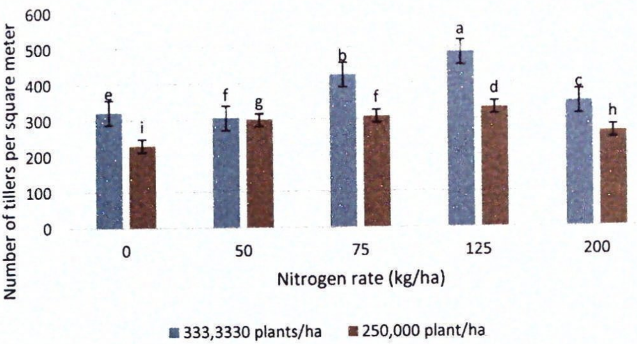

Figure 4.32: Interactive effect of plant density and nitrogen application rate on number of tillers per square meter during 2016

4.11.2 Effect of plant density and nitrogen application rate on number of spikelets per spike

During the 2015 season, plant density and nitrogen application rate did not interact significantly to influence the number of spikelets per spike of wheat, but did so only in 2016 (Figure 4.33). At both plant densities, more spikelets per spike were obtained when plants received 125 kg/ha of N, but, there were no significant differences between the two densities.

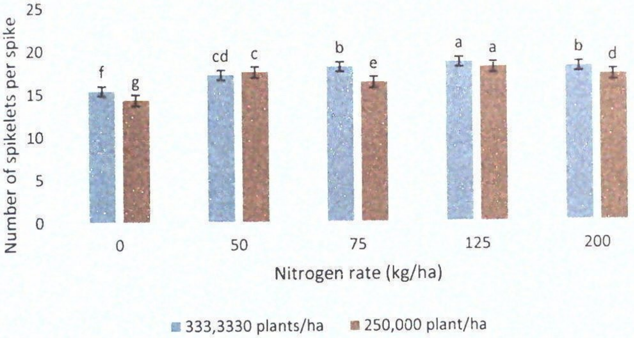

Figure 4.33: Interactive of plant density and nitrogen application rate on number of spikelets per spike during 2016

4.11.3 Effect of plant density and nitrogen application rate on number of grains per spike

Plant density and nitrogen significantly enhanced the number of grains per spike of wheat during the 2015 season (Figure 4.34). The highest number of grains per spike was recorded at 125 kg/ha of N and 333,333 plants/ha significantly ($p \leq 0.05$) being different from the 250,000 plants/ha density. This trend was also evident in 2016 with the density of 333,333 producing more grains per spike at the N rate of 125 kg/ha (Figure 4.35).

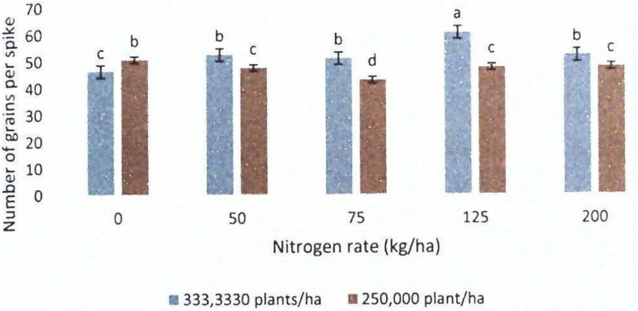

Figure 4.34: Interactive of plant density and nitrogen application rate on number of grains per spike during 2015

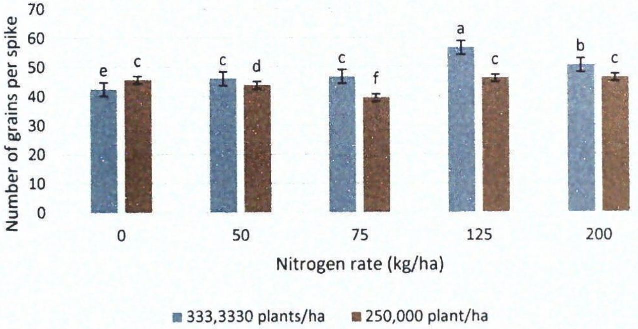

Figure 4.35: Interactive effect of plant density and nitrogen application rate on number of grains per spike during 2016

4.11.4 Effect of plant density and nitrogen application rate on biological yield of wheat

Figure 4.36 shows the influence of plant density and nitrogen on biological yield of wheat during 2015. The highest biological yield was produced by plants that received 125 kg/ha of N at both densities. In 2016, however, there were differences in biological yield between all the densities except at 125 kg/ha of N (Figure 4.37).

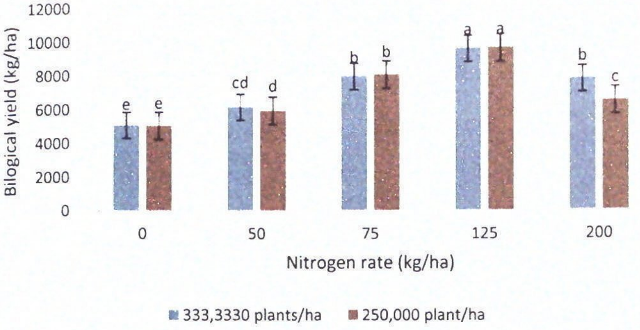

Figure 4.36: Interactive effect of plant density and nitrogen application rate on biological yield during 2015

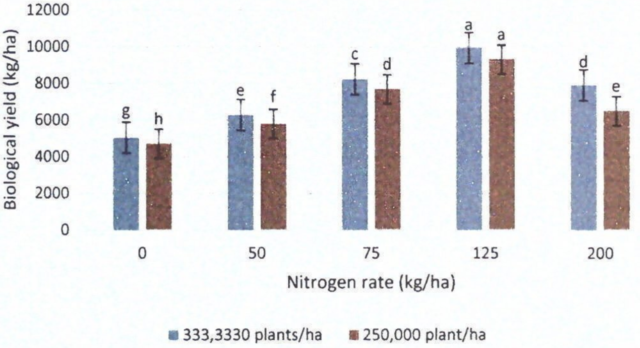

Figure 4.37: Interactive effect of plant density and nitrogen application rate on biological yield during 2016

4.11.5 Effect of plant density and nitrogen application rate on grain yield of wheat

Plant density and nitrogen interacted significantly and enhanced grain yield of wheat during 2015 as reflected in Figure 4.38. However, there were no significant differences in yield between the two densities. The highest value of grain yield was recorded at N of 125 kg/ha. A similar pattern was observed in 2016, where 125 kg/ha of N produced the highest grain similarly for both densities (Figure 4.39).

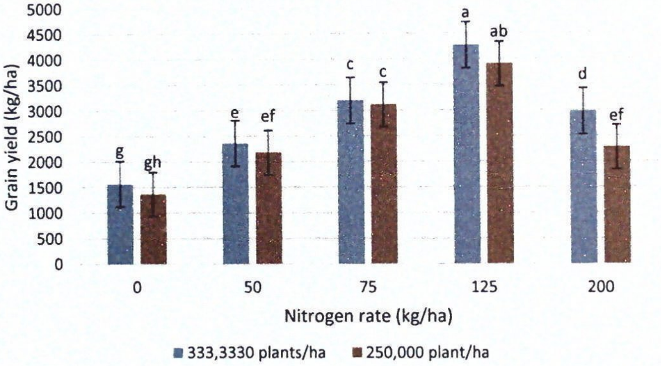

Figure 4.38: Interactive effect of plant density and nitrogen application rate on grain yield of wheat during 2015

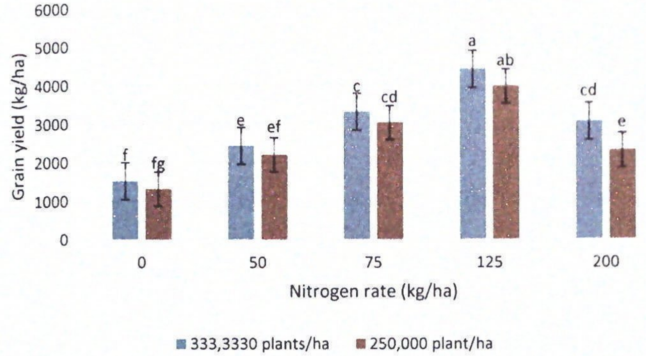

Figure 4.39 Interactive effect of plant density and nitrogen application rate on grain yield of wheat during 2016

4.12 Effect of plant density, planting date and genotype interaction on wheat

4.12.1 Interactive effect of plant density, planting date and genotype on the number of tillers

In 2015, plant density, planting date, and genotype interacted to influence the number of wheat tillers. Both wheat genotypes produced similar number of tillers during the 21st April planting at 333,333 plants per hectare at 401.8 tillers/m^2. Also, more tillers were recorded under the higher plant density compared to the lower one for all genotype planting date combinations (Figure 4.40).

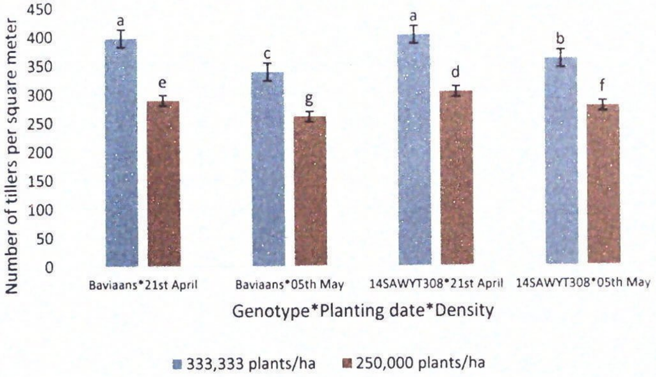

Figure 4.40 Interactive effect of plant density, planting date, and genotype on number of tillers per square meter during 2015

4.12.2 Effect of plant density, planting date and genotype on number of spikelets per spike

There were no significant differences between the numbers of spikelets/spike produced by the two varieties when planted on 21st April using the higher planting density during 2015 (Figure 4.41). For the genotype Baviaans, during the second planting date, plant density did not affect the number of spikelets/spike. Regarding the 14SAWYT308 genotype, the 21st April planting date exhibited significantly higher number of spikelets/spike compared to late planting. Furthermore, for 14SAWYT308, more spikelets/spike were produced under the first planting date compared to the second one.

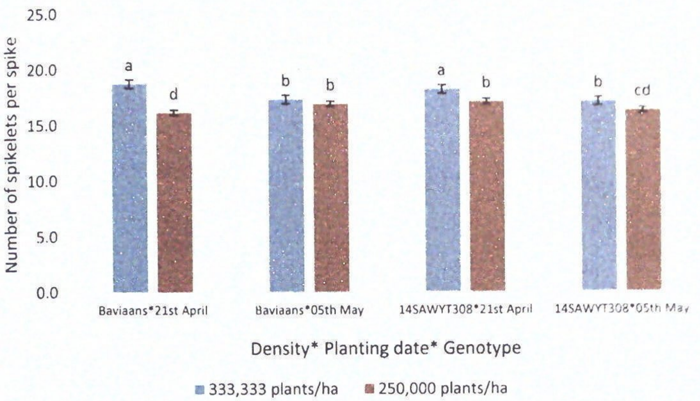

Figure 4.41: Interactive effect of plant density, planting date and genotype on number of spikelets per spike during 2015

4.12.3. Effect of plant density, planting date and genotype on number of grains per spike of wheat

Figure 4.42 shows the effect of plant density, planting date, and genotype on number of grains per spike of wheat during 2015. Both wheat genotypes exhibited the highest number of grains per spike when planted on 21st April at the higher density of 333,333 plants/ha. Generally, wheat planted at the higher density irrespective of planting date, produced more grain per spike compared to the lower density except for combination of 14SAWYT308 during the 05th May date. In 2016, the 14SAWYT308 when planted earlier at the higher density, produced significantly more grain per spike compared to the Baviaans under similar conditions (Figure 4.43). Similar to the 2015 case, in 2016, wheat planted at 333,333 exhibited higher grains per spike relative to the lower density, except at the 05th May planting.

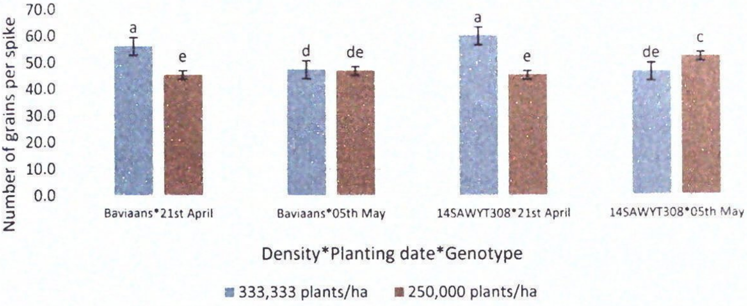

Figure 4.42: Interactive effect of plant density, planting date, and genotype on number of grains per spike during 2015

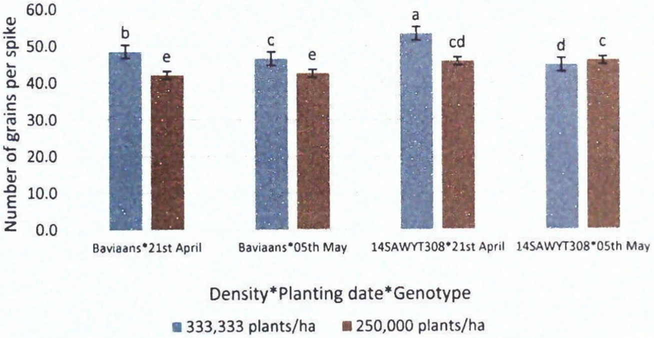

Figure 4.43: Interactive effect of plant density, planting date, and genotype on number of grains per spike during 2016

4.12.4 Effect of plant density, planting date, and genotype on biological yield of wheat

Plant density, planting date and wheat genotype interacted significantly to influence biological yield as shown in Figure 4.44. The 14SAWYT308 genotype performed better than Baviaans when planted at 333,333 plants per hectare during the 21st April planting. For both genotypes, the higher density produced more biological yield compared to the lower one during first planting but not during 05th May planting. For the 2015 season interaction of density, planting date and genotype had no significant effect on biological yield of wheat.

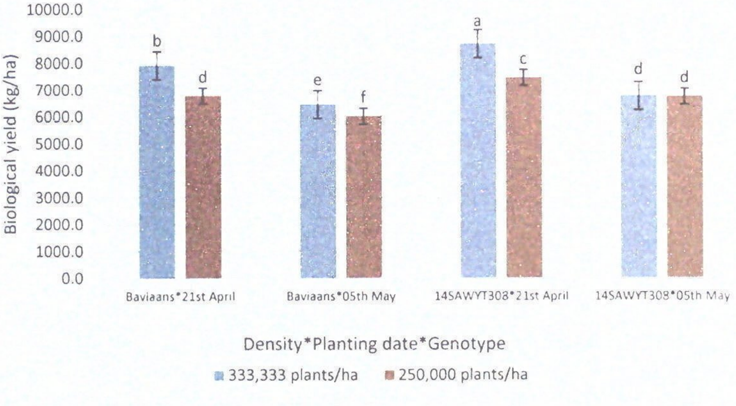

Figure 4.44: Interactive effect of plant density, planting date, and genotype on biological yield during 2016

4.12.5 Effect of plant density, planting date, and genotype on grain yield of wheat

The effect of plant density, planting date and genotype on grain yield of wheat during 2015 is shown in Figure 4.45. Genotype 14SAWYT308 from 21st April planting date, at plant density of 333,333 plants/ha produced highest grain yield, significantly different from 250,000 plants/ha density for the same genotype and same planting date. Plant density, planting date, and genotype interaction did not significantly enhance grain yield for both genotype, at 05th May planting date. A similar trend was observed during 2016 whereby 14SAWYT308 genotype from first planting date at 333,333 plants/ha produced maximum grain yield (Figure 4.46).

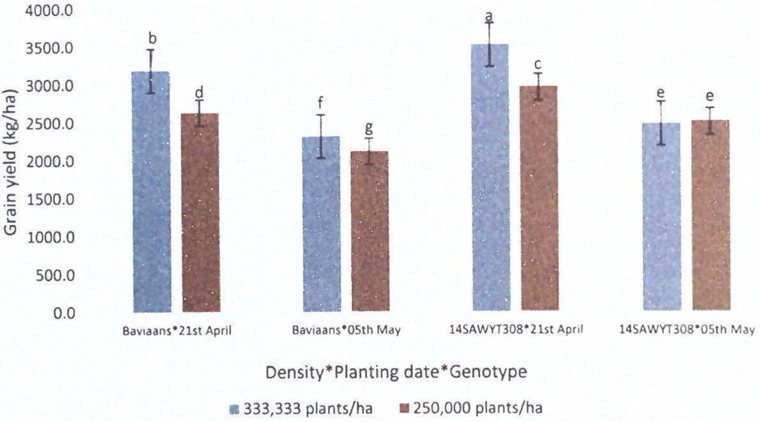

Figure 4.45: Interactive effect of plant density, planting date, and genotype on grain yield during 2015

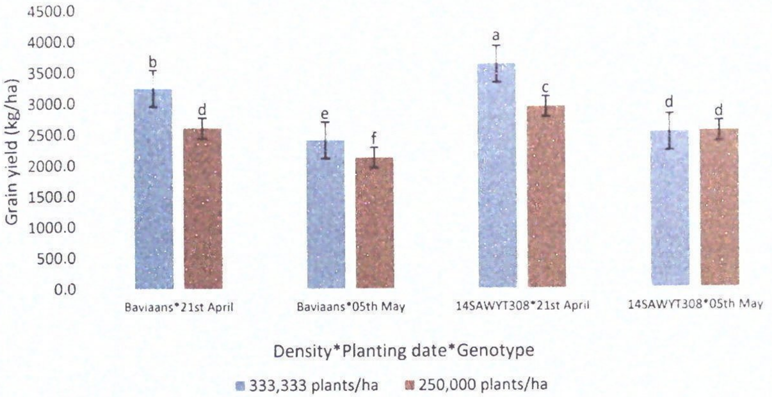

Figure 4.46: Interactive effect of plant density, planting date, and genotype on grain yield during 2016

4.13 Effect of nitrogen application rate and planting date interaction on wheat

4.13.1 Effect of nitrogen and planting date on number of tillers

Figure 4.47 shows the effect of nitrogen application rate and planting date on number of tillers of wheat during 2015 season. Significantly more tillers were produced during the 21st April planting treatment at 75 kg/ha of N and at 125 kg/ha of N compared to 05th May planting and other N levels. A similar trend was noted in 2016. Also, during this year, there were significant differences between the number of tillers produced at 75 kg/ha of N and 125 kg /ha of N, with the latter producing more tillers (Figure 4.48).

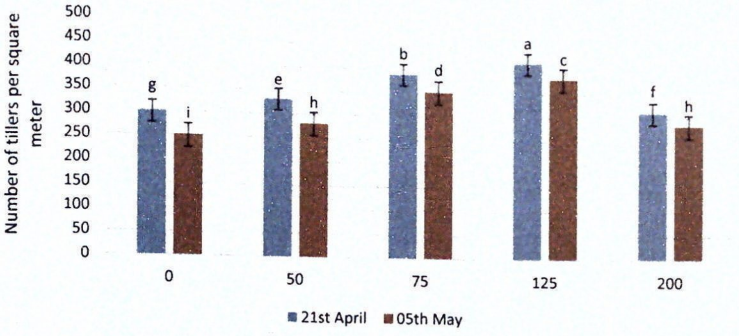

Figure 4.47: Interactive effect of nitrogen and planting date on number of tillers during 2015

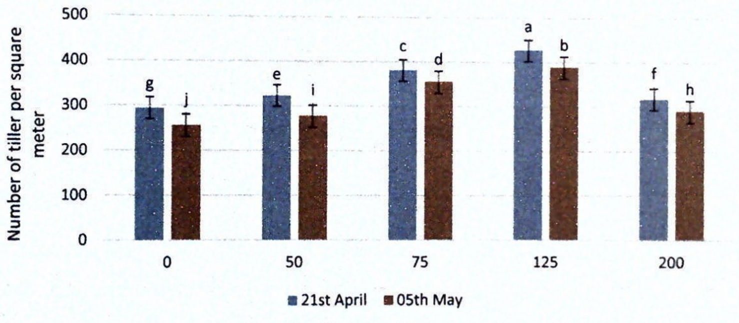

Figure 4.48: Interactive effect of nitrogen and plant date on number of tillers during 2016

4.13.2 Effect of nitrogen and planting date on number of spikelets per spike of wheat

Nitrogen application and planting date did not affect the number of spikelets/spike of wheat between the two planting dates except at the application rates of 50 and 200 kg/ha of N during 2015 season (Figure 4.49). Furthermore, the highest number of spikelets/spike were exhibited by plants that received 50 kg/ha of N during 21st April planting, 125 kg/ha of N irrespective of planting date and 200 kg/ha of N under earlier planting. During 2016 season the interaction of nitrogen application rate and planting date on number of spikelets per spike was not statistically significant.

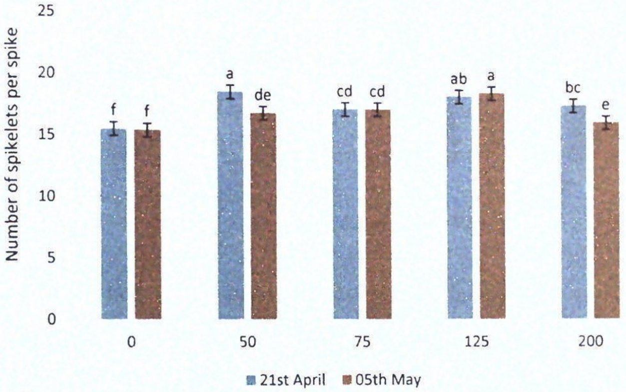

Figure 4.49: Interactive effect of nitrogen and planting date on number of spikelets per spike during 2015

4.13.3 Effect of nitrogen and planting date on the number of grains per spike of wheat

Figure 4.50 shows the effect of nitrogen application and planting date on the number of grains per spike of wheat in 2016. The interaction of 125 kg/ha of N and 21st April planting produced the highest number of grains per spike, significantly different from mean grains per spike obtained from interaction of 125 kg/ha of N and 05th May planting date. The least number of grains per spike was recorded from the nitrogen rate of 75 kg/ha and 21st April planting date interaction. During 2015 season the interaction of nitrogen and planting date had no significant effect on the number of grains per spike.

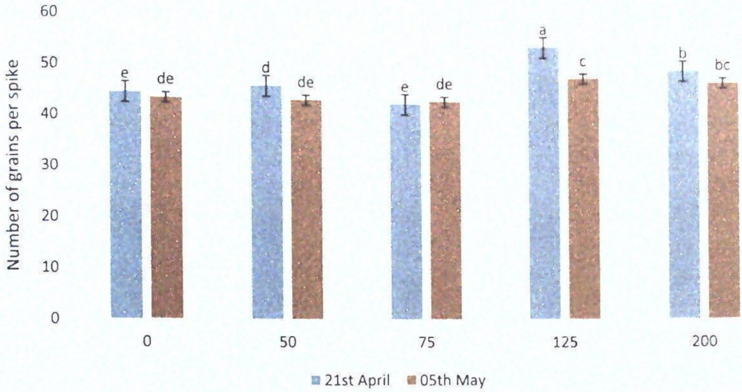

Figure 4.50: Interactive effect of nitrogen and planting date on number of grains per spike during 2016

4.13.4 Effect of nitrogen and planting date on biological yield of wheat

The interactive effects of nitrogen rate and planting date on biological yield of wheat during 2015 are shown in Figure 4.51. Plants supplied with 125 kg/ha of nitrogen and grown on first planting date gave highest biological yield, and the mean was significantly different from that of plants that received 125 kg/ha of N but grown on 05th May.

A similar pattern was observed in 2016, where the highest mean biological yield was recorded from 125 kg/ha of N and 21st April planting (Figure 4.52).

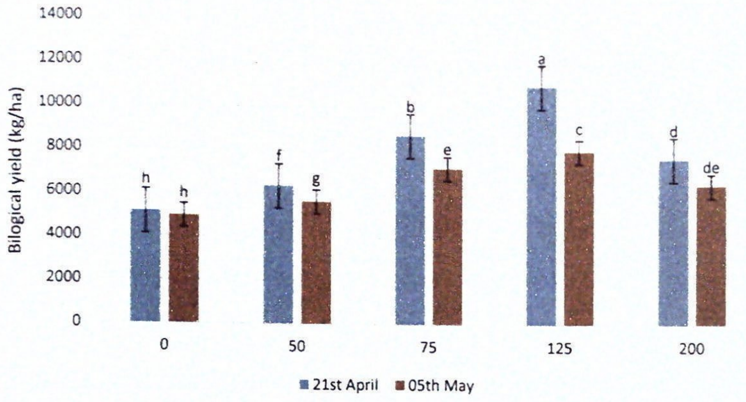

Figure 4.51: Interactive effect of nitrogen and planting date on biological yield of wheat during 2015

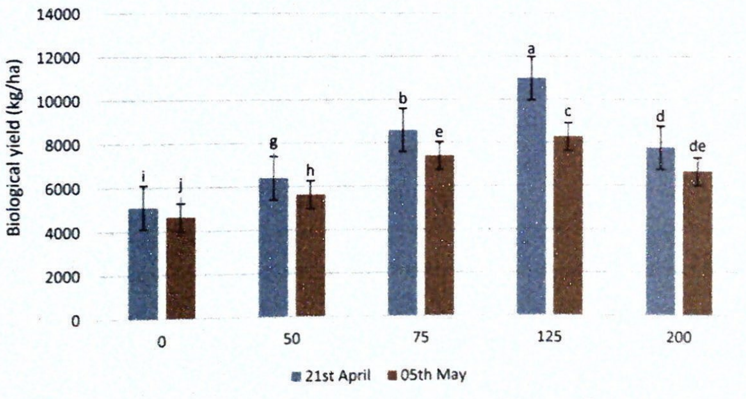

Figure 4.52: Interactive effect of nitrogen and planting date on biological yield of wheat during 2016

4.13.5 Effect of nitrogen and planting date on grain yield of wheat

The effect of nitrogen application and planting date interaction on grain yield of wheat in 2015 is shown in Figure 4.53. Crops that were planted on 21st April and received 125 kg/ha of nitrogen gave highest the grain yield which was significantly different from that of those planted on 05th May and received 125 kg/ha of nitrogen. The least grain yield was obtained from 0 kg/ha nitrogen rate and both planting dates. Similarly in 2016, the highest mean grain yield was obtained from 125 kg/ha of N and 21st April planting date interaction, and that mean significantly differed from that of 125 kg/ha of N and 05th May planting date interaction (Figure 4.54).

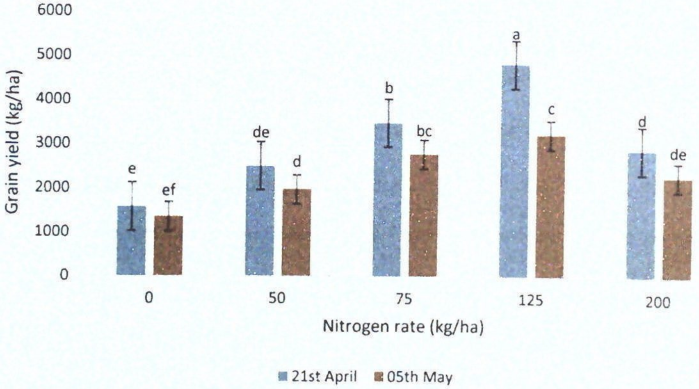

Figure 4.53: Interactive effect of nitrogen and planting date on grain yield of wheat during 2015

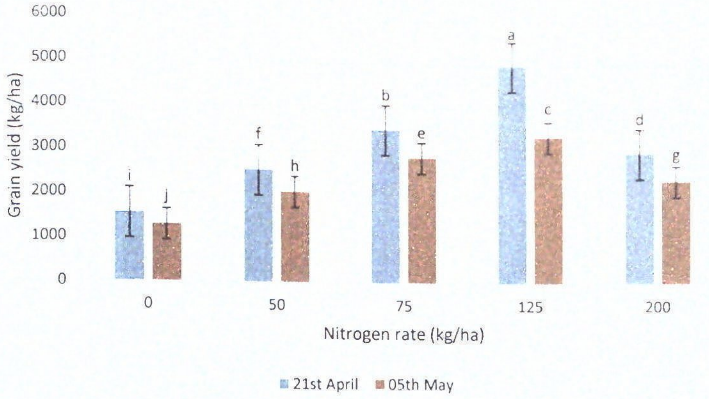

Figure 4.54: Interactive effect of nitrogen and planting date on grain yield of wheat during 2016

4.14 Effect of nitrogen, plant density and genotype interaction on wheat

4.14.1 Effect of nitrogen, planting density and genotype on the number of wheat tillers

Figure 4.55 shows the effect of nitrogen rate, plant density and genotype interaction on number of tillers of wheat during 2016. Both genotypes produced the highest number of tillers per square metre when given 125 kg/ha of N at plant density of 333,333 plants/ha. In 2015 the interaction of nitrogen rate, plant density and genotype on number of tillers was not statistically significant.

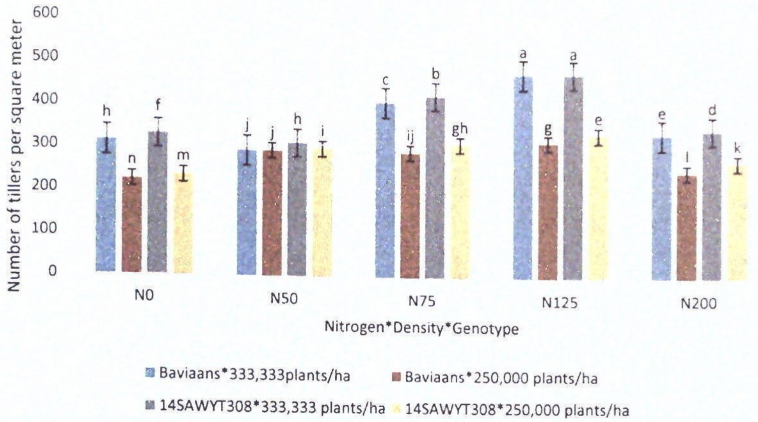

Figure 4.55: Interactive effect of nitrogen, planting density and genotype on number of tillers during 2016.

4.14.2 Effect of nitrogen, planting density and genotype on number of spikelets per spike

The effect of nitrogen application rate, plant density and genotype interaction on the number of spikelets per spike during 2015 is shown in Figure 4.56 Genotype 14SAWYT308 fertilized with 50 kg/ha of N at density of 333,333 plants/ha gave the highest mean number of spikelets per spike and was significantly different from those planted at density of 250,000 plants/ha. However in 2016, unlike in 2015, 14SAWYT308 genotype plants with 75 and 125 kg/ha of N at 333,333 plants/ha had the highest number of spikelets/spike. Furthermore, Baviaans plants that received 200 kg/ha of N and planted at 333,333 plants/ha also produced similar number of spikelets/spike (Figure 4.57).

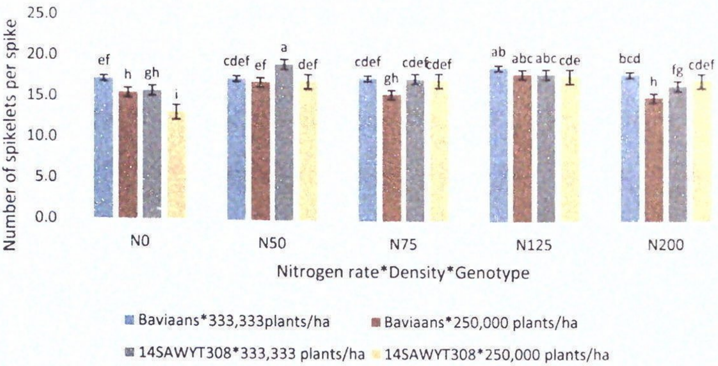

Figure 4.56: Interactive effect of nitrogen, planting density and genotype on number of spikelets per spike during 2015

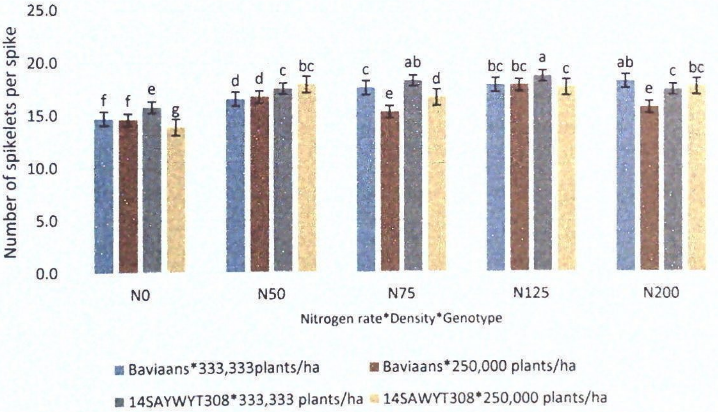

Figure 4.57: Interactive effect of nitrogen, planting density and genotype on number of spikelets per spike during 2016

4.14.3 Effect of nitrogen, planting density and genotype on number grains per spike

Figure 4.58 depicts the interactive effects of nitrogen rate, plant density and genotype on the number of grains/spike of wheat during 2015. Baviaans genotype provided with 125 kg /ha of

N at 333,333 plants/ha produced highest number of grains per spike and the mean significantly differed from other treatments.

Results showed that during 2016 the highest grains per spike mean was recorded for both genotypes at N rate of 125 kg/ha and density of 333,333 plants/ha (Figure 4.59).

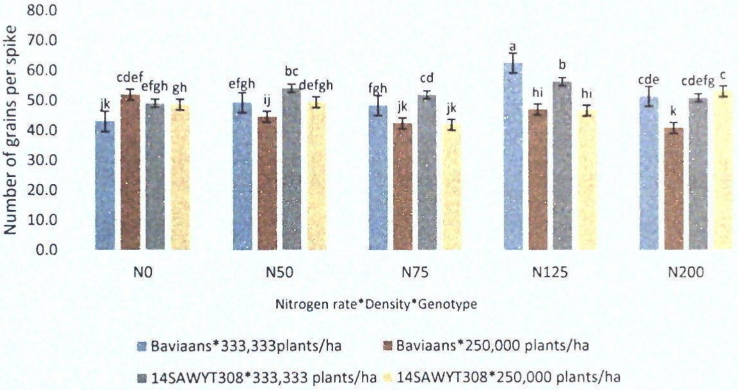

Figure 4.58: Interactive effect of nitrogen, planting density and genotype on number grains per spike during 2015

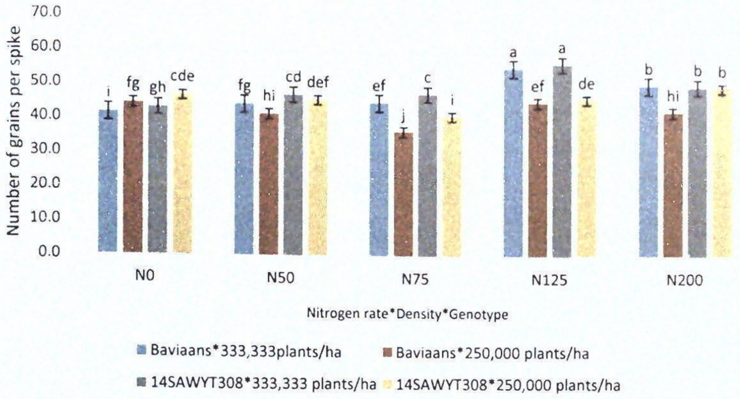

Figure 4.59: Interactive effect of nitrogen, planting density and genotype on number grains per spike during 2016

4.14.4 Effect of nitrogen, planting density and genotype on biological yield of wheat

Interactive effect of nitrogen application rate, plant density and genotype on biological yield of wheat during 2015 are shown on Figure 4.60. Significantly higher biological yield was recorded from genotype 14SAWYT308 with 125 kg/ha of N at 250,000 plants/ha interaction significantly different from Baviaans with 125 kg/ha of N at 333,333 plants/ha, Baviaans with 125 kg/ha of N at 250,000 plants/ha and 14SAWYT308 with 125 kg/ha of N at 333,333 plants/ha. Minimum mean biological yield was obtained from Baviaans genotype at 0kg/ha nitrogen rate with 333,333 plants/ha interaction, and the mean statistically different from that of 14SAWYT308 with 0 kg/ha of N with 333,333 plants/ha and 14SAWYT308 with 0 kg/ha of N at 250,000 plants/ha interaction but not Baviaans with 0 kg/ha of N at 250,000 plants/ha. Consistently for 2016 season highest biological yield was recorded from genotype 14SAWYT308 with 125 kg/ha of N at 250,000 plants/ha interaction but significantly different from only interaction of Baviaans with 125 kg/ha of N at 250,000 plants/ha (Figure 4.61).

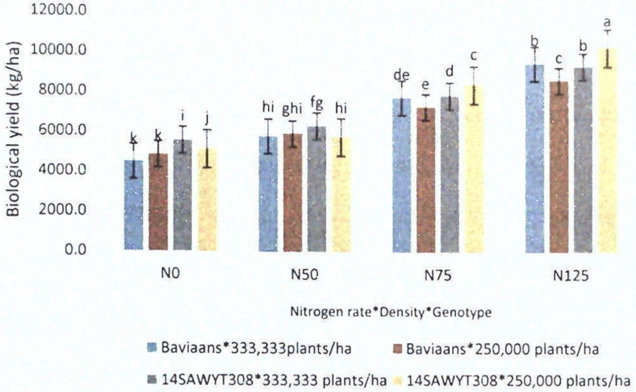

Figure 4.60: Interactive effect of nitrogen, planting density and genotype on biological yield of wheat during 2015

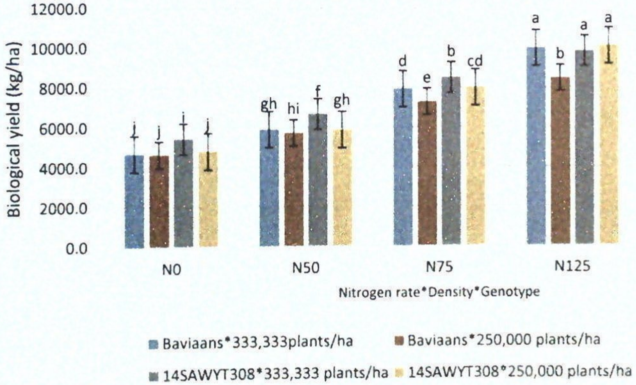

Figure 4.61: Interactive effect of nitrogen, planting density and genotype on biological yield of wheat during 2016

4.14.5 Effect of nitrogen, planting density and genotype on grain yield of wheat

Nitrogen application rate, plant density and genotype interaction significantly influenced grain yield of wheat during 2015 season (Figure 4.62). Baviaans genotype applied with 125 kg/ha of nitrogen fertilizer, and planted at density of 333,333 plants/ha produced highest grain yield significantly higher than interaction of Baviaans with 125 kg/ha of N at 250,000 plants/ha density, and 14SAWYT308 with 125 kg/ha of N at 333,333 plants/ha. The minimum grain yield of was observed from Baviaans genotype at 0 kg/ha with 250,000 plants/ha density, significantly different from interaction of Baviaans with 0 kg/ ha of N at 333,333 plants/ha, 14SAWYT308 with 0 kg/ ha of N at 333,333 plants/ha and 14SAWYT308 with 0 kg/ha of N at 250,000 plants/ha.

Data from 2016 season revealed a consistent pattern where maximum grain yield was obtained from Baviaans fertilised with 125 kg/ha of N fertilization at 333,333 plants/ha interaction and least grain yield mean realized from Baviaans genotype with 0 kg/ha of N, at 250,000 plants/ha interaction (Figure 4.63).

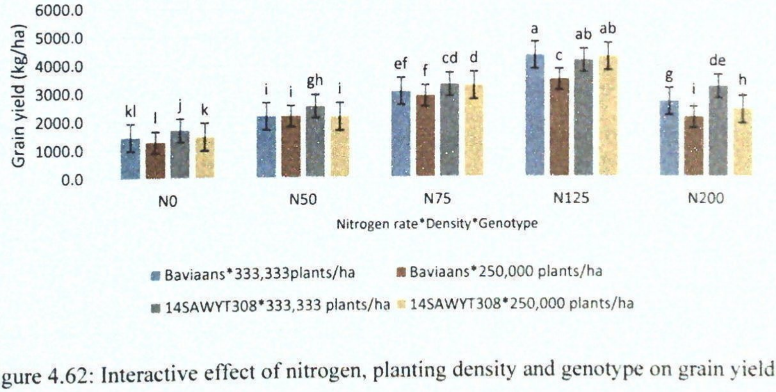

Figure 4.62: Interactive effect of nitrogen, planting density and genotype on grain yield of wheat during 2015

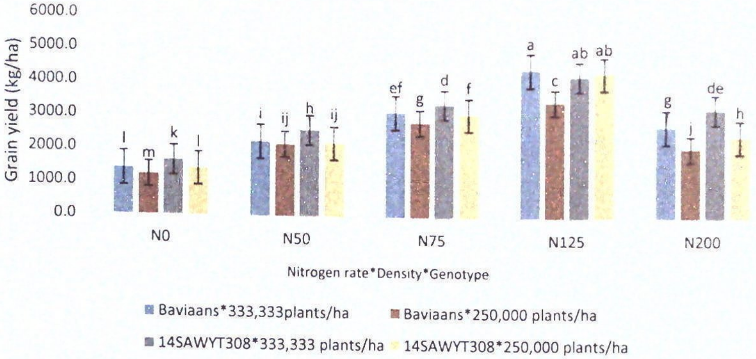

Figure 4.63: Interactive effect of nitrogen, planting density and genotype on grain yield of wheat during 2016

4.15 Effect of planting date, plant density and nitrogen rate on wheat

4.15.1 Effect of planting date, planting density and nitrogen on the number of tillers

Figure 4.64 showed that the number of tillers of wheat were significantly influenced by the interaction of planting date, plant density and nitrogen rate during 2015 season. The highest number of tillers was obtained from crops of 21st April planting date, at density of 333,333 plants/ha with nitrogen application of 125 kg/ha added to them. The mean significantly differed with mean obtained from crops at 05th May planting date, 333,333 plants/ha density, and nitrogen dose of 125 kg/ha significantly differing from interaction of 21st April planting date crops at 250,000 plants/ha with 125 kg/ha of N, and 05th May planting date at 250,000 plants/ha with 125 kg/ha of N. A similar trend occurred in 2016 where the highest number of tillers were produced from 21st April planting, 333,333 plants/ha density and 125 kg/ha of N interaction, while least number of tillers were recorded at 05th May planting date, 250,000 plants/ha density and 0 kg/ha of N (Figure 4.65).

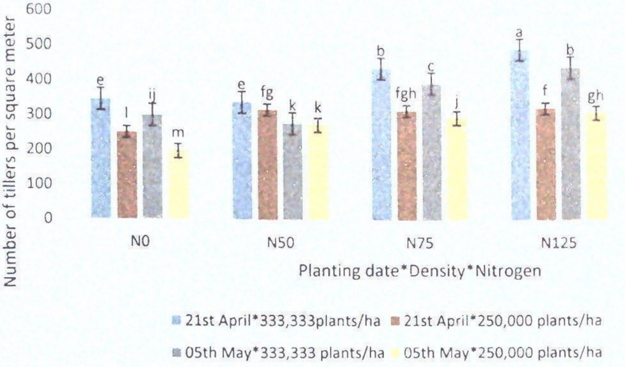

Figure 4.64: Interactive effect of planting date, planting density and nitrogen on number of tillers during 2015

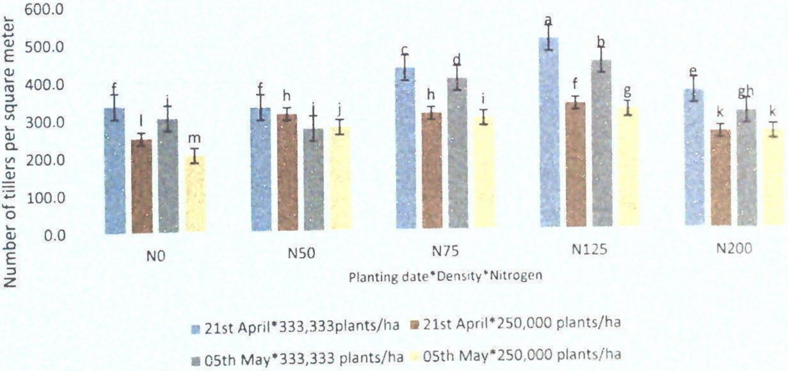

Figure 4.65: Interactive effect of planting date, planting density and nitrogen on number of tillers during 2016

4.15.2 Effect of planting date, planting density and nitrogen on number of spikelets per spike

The interaction of planting date, plant density and nitrogen application rate significantly affected number of spikelets per spike of wheat during 2015 season (Figure 4.66). The highest number of spikelets per spike was observed at plants grown on 21st April, at density of 333,333 plants/ha and supplied with 125 kg/ha of nitrogen. The mean significantly differed with that of crops supplied with same 125 kg/ha of N fertilizer at 21st April with density of 250,000 plants/ha, crops from 05th May with 333,333 plants/ha and 05th May with 250,000 plants/ha density. Consistently in 2016, highest number of spikelets per spike was obtained at interaction of 21st April planting date, 333,333 plants/ha density and 125 kg/ha of N while least number of spikelets per spike was recorded in 05th May planting date, 250,000 plants/ha density, and no nitrogen rate interaction (Figure 4.67).

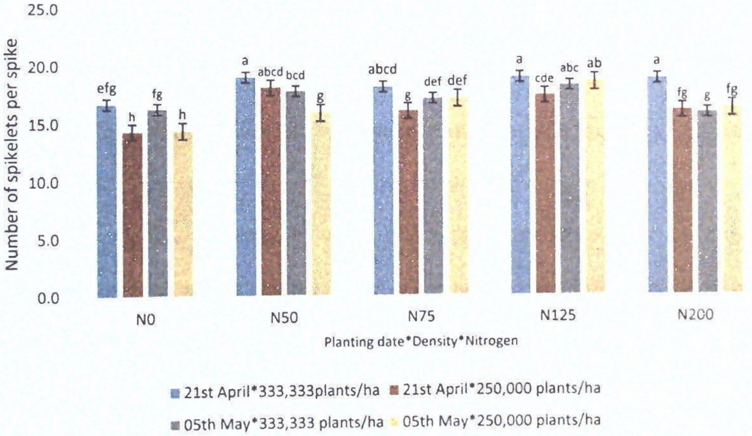

Figure 4.66: Interactive effect of planting date, planting density and nitrogen on number of spikelets/spike during 2015

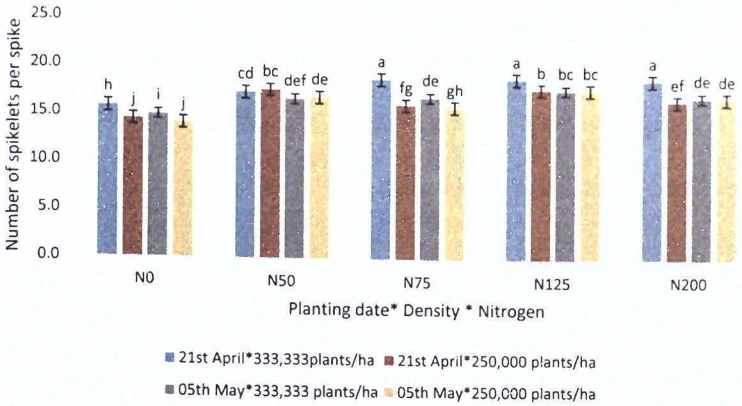

Figure 4.67: Interactive effect of planting date, planting density and nitrogen on number of spikelets per spike during 2016

4.15.3 Effect of planting date, planting density and nitrogen on number of grains per spike

The number of grains per spike of wheat was significantly affected by the interaction of planting date, plant density and nitrogen application rate during the 2015 season (Figure 4.68). Plants grown on 21st April, density of 333,333 plants/ha and 125 kg/ha of nitrogen gave highest number of grains per spike, which statistically differed with that of crops given same 125 kg/ha of N on 21st April at 250,000 plants/ha, 05th May crops at 333,333 plants/ha with 125 kg/ha of N, and 05th May at 250,000 plants/ha with 125 kg/ha of N interaction. Least number of grains per spike was obtained from crops grown on 21st April, at 250,000 plants/ha and supplied with 75 kg/ha of nitrogen fertilizer. The mean significantly differed from crops of 21st April at 333,333 plants/ha with 75 kg/ha of N, crops from 05th May at 333,333 plants/ha with 75 kg/ha of N and 05th May at 250,000 plants/ha with 75 kg/ha of N.

Likewise, for 2016 maximum number of grains per spike was obtained at 21st April planting, 333,333 plants/ha and 125 kg/ha of N interaction, but minimum number of grains per spike was consistently obtained from 21st April planting date, 250,000 plants/ha and 75 kg/ha of N (Figure 4.69).

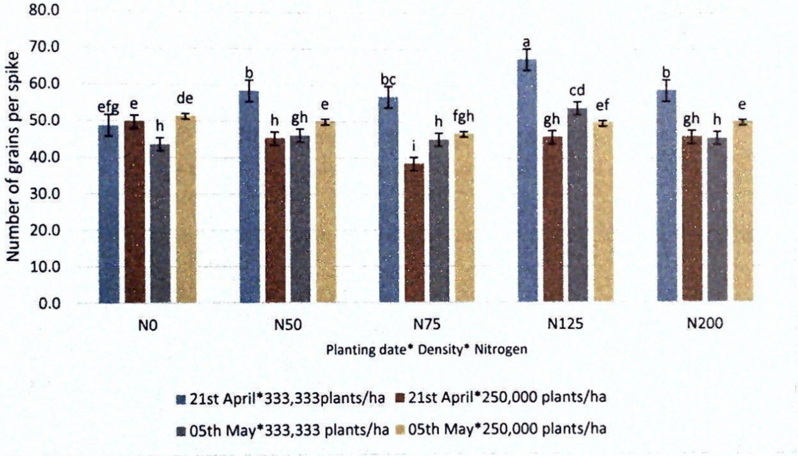

Figure 4.68: Interactive effect of planting date, planting density and nitrogen on number of grains per spike during 2015

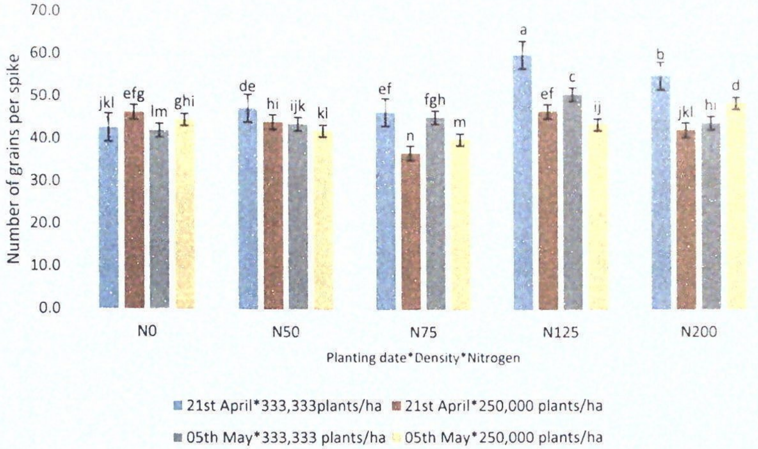

Figure 4.69: Interactive effect of planting date, planting density and nitrogen on number of grains per spike during 2016

4.15.4 Effect of planting date, planting density and nitrogen on biological yield of wheat

Effect of planting date, plant density and nitrogen application rate on biological yield of wheat is reflected on Figure 4.70. The highest biological yield was recorded on 21st April, at 333,333 plants/ha and given 125 kg/ha of N. The mean was statistically different from those of plants given 125 kg/ha of N nitrogen on 21st April with 250,000 plants/ha density, plants from 05th May at 333,333 plants/ha with 125 kg/ha of N, and 05th May plants at 250,000 plants/ha with 125 kg/ha of N. A similar pattern occurred in 2016 where the highest biological yield was recorded at 21st April plants at 333,333 plants/ha density and applied 125 kg/ha of nitrogen (Figure 4.71).

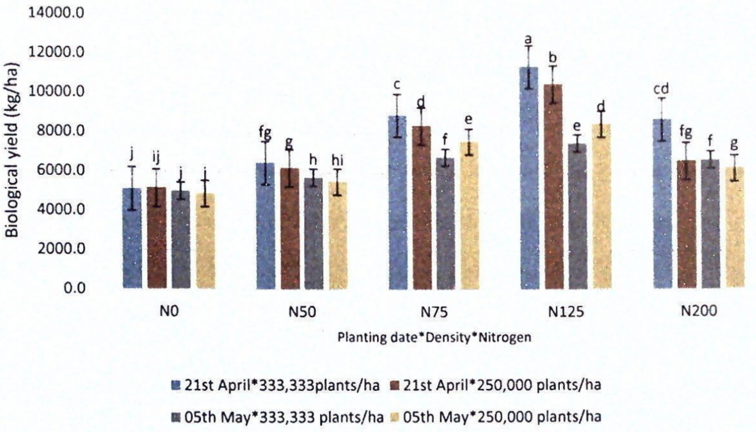

Figure 4.70: Interactive effect of planting date, planting density and nitrogen on biological yield of wheat during 2015

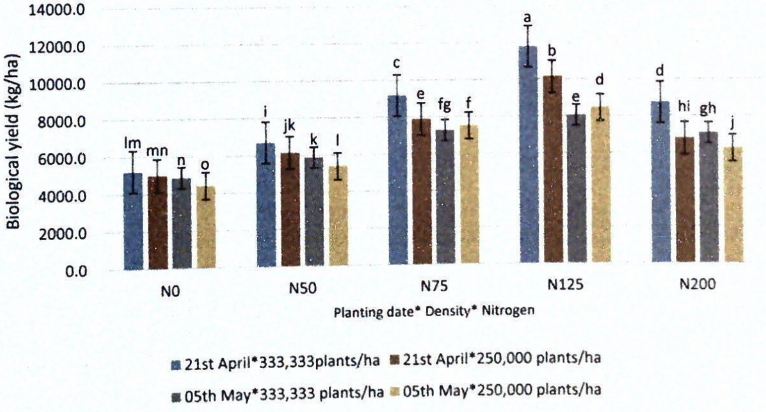

Figure 4.71: Interactive effect of planting date, planting density and nitrogen on biological yield of wheat during 2016

4.15.5 Effect of planting date, planting density and nitrogen on grain yield of wheat

Interactive effects of planting date, plant density and nitrogen application rate on grain yield of wheat during 2015 is shown on Figure 4.72. The plants from 21st April density of 333,333 plants/ha and applied 125 kg/ha of nitrogen fertilizer gave highest grain yield. The mean significantly differed from that of other treatments. Similarly in 2016 the highest grain yield was obtained from 21st April, 333,333 plants/ha, 125 kg/ha of N interaction, significantly higher than mean from 21st April, 250,000 plants/ha, 125 kg/ha of N interaction, 05th May planting, 250,000 plants/ha, 125 kg/ha of N interaction, and 05th May, 333,333 plants/ha, 125 kg/ha of N interaction (Figure 4.73).

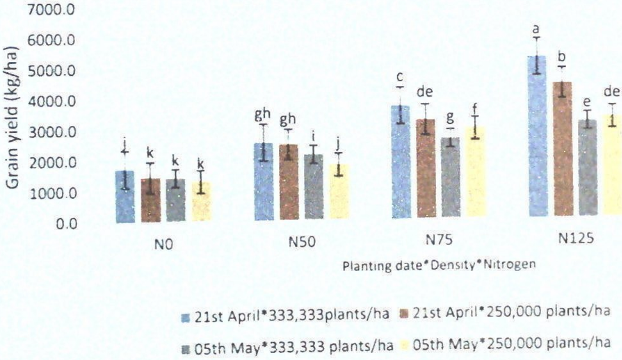

Figure 4.72: Interactive effect of planting date, planting density and nitrogen on grain yield of wheat during 2015

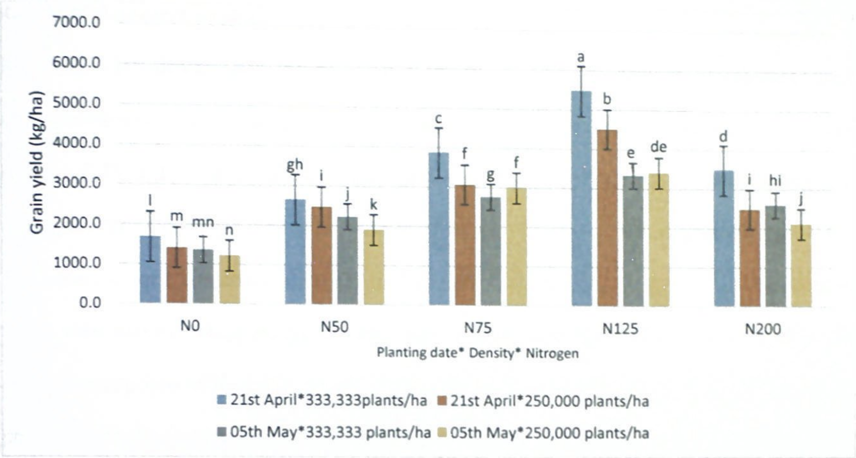

Figure 4.73: Interactive effect of planting date, planting density and nitrogen on grain yield of wheat during 2016

4.16 Wheat protein profiles

Figure 4.74 is a dendrogram of two wheat genotypes showing protein size similarities based on 40 treatments (Y-axis), using Un-weighted Pair Group Method with Arithmetic mean cluster analysis (UPGMA). There were four main clusters of nitrogen, plant density, and planting date interaction treatments (labelled 1, 2, 3 and 4) demarcated at coefficient of approximately 0.63. The main cluster 1 consisted of treatments $N_{50}Dn_1Dt_2V_2$, and $N_{75}Dn_2Dt_2V_1$, where the protein bands were similar. Main cluster 2 comprised of treatments $N_{75}Dn_1Dt_1V_2$ only, while main cluster 4 comprised of $N_0Dn_1Dt_1V_2$ and $N_{50}Dn_1Dt_1V_2$, and likewise, they showed similarity in protein bands. Most of the plant density, nitrogen rate and planting date treatments interactions were found under main cluster 3, comprising 35 combinations of nitrogen, plant density, and planting date interaction treatments (Figure 4.74).

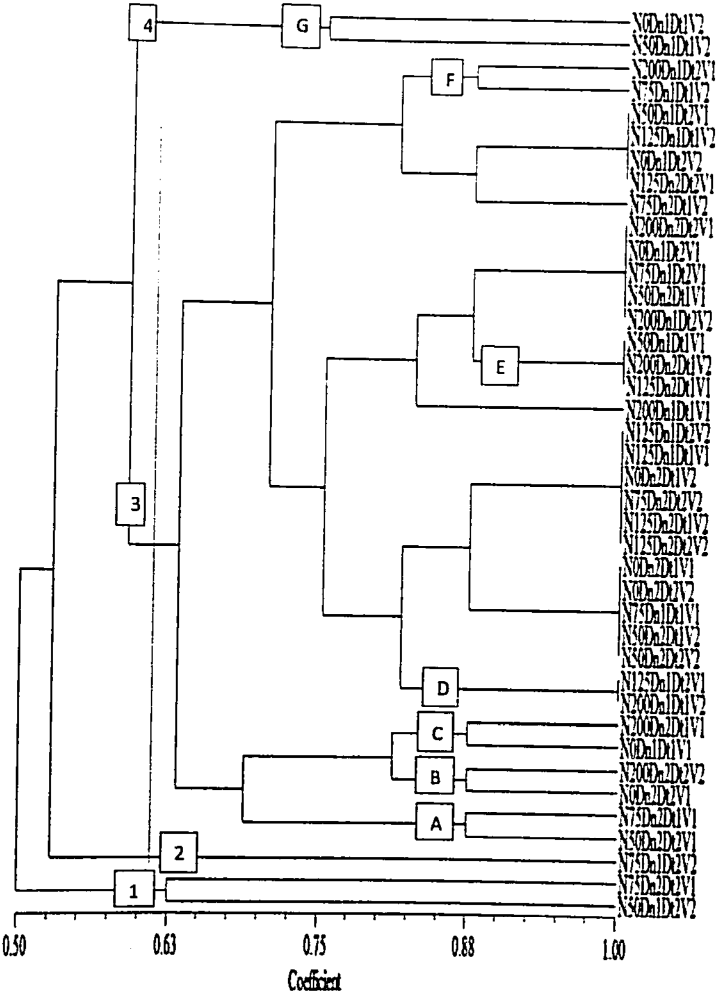

Figure 4.74: Dendrogram of two wheat genotypes showing protein size similarities based on 40 treatments (Y-axis), using UPGMA cluster analysis. The X-axis shows similarity coefficients between treatments. Labels 1 to 4 shows main clusters demarcated at 0.63 coefficient.

Table 4.6 shows Eigen vectors, Eigen values and first four Principal Component Axes in protein band sizes of wheat genotypes. The principal component analysis revealed protein bands sizes which accounted for variances. The four principal components depicted an accumulated total variation of 72.92%. However, Principal Component 1 (PC 1) accounted for most variability (29.17%), with most contributions obtained from protein band sizes of 22 kDa (-0.8894), 32 kDa (-0.8670) and 58 kDa (-0.8894). Principal Component 2 loaded mostly 17 kDa (-0.8660) and 75 kDa (-0.8660) which also added to variation in band sizes.

Table 4.6: Eigen vectors, Eigen values, Proportion and Cumulative % of variability for the first four principal components among protein band sizes of two wheat genotypes

Band size (kDa)	PC 1	PC 2	PC 3	PC 4
17	0.0000	-0.8660	0.0000	0.0000
22	-0.8894	0.0000	0.0000	0.0000
25	0.0000	0.0000	1.0000	0.0000
32	-0.8670	0.0000	0.0000	0.0000
46	0.0000	0.0000	0.0000	1.0000
58	-0.8894	0.0000	0.0000	0.0000
75	0.0000	-0.8660	0.0000	0.0000
Eigen value/latent root	2.3336	1.5000	1.0000	1.0000
Proportion (%)	29.17	18.75	12.50	12.50
Cumulative %	29.17	47.92	60.42	72.92

Figures 4.75 to 4.78 show some of the electrophoregrams depicting banding patterns of molecular weight marker and wheat proteins from genotypes subjected to different treatments of nitrogen, plant density, and planting date.

On Figure 4.75, the treatments loaded in the gel were genotype 14SAWYT308 from second planting date at density of 333,333 plants/ha and given 50 kg/ha of nitrogen fertilizer ($N_{50}Dn_1Dt_2V_2$) (first three replications), which is a representative of main cluster 1. Binary data matrix analysis showed that protein bands from the gel contained bands of molecular weight 11 kDa, 17kDa, 32 kDa and 58 kDa.

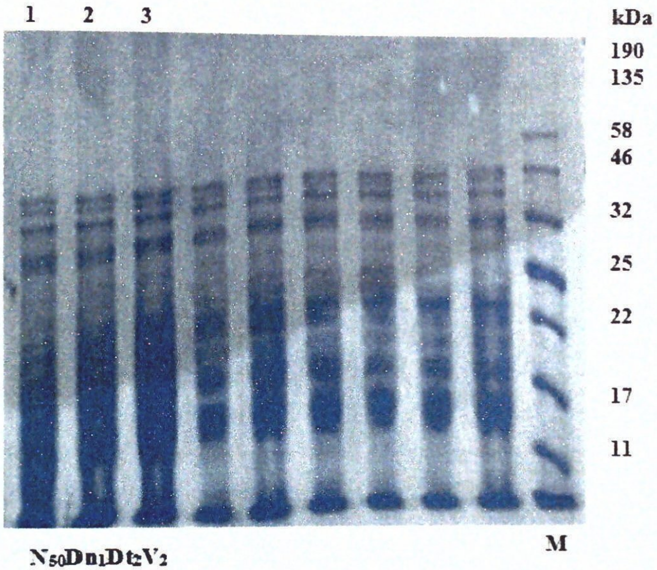

Figure 4.75: One dimensional SDS-PAGE separation of proteins of wheat genotype 14SAWYT308. Molecular weight maker was represented by label M. while 1, 2, and 3 represents sample replications in the gel

In Figure 4.76, three treatments of nitrogen, plant density and planting date were casted in the gel. In the first three wells of the gel, labelled 1, 2, and 3 is genotype 14SAWYT308 from the second planting date at a density of 333,333 plants/ha and 75 kg/ha N fertilizer ($N_{75}Dn_1Dt_2V_2$), representative of cluster 2. According to binary data matrix analysis protein bands from electrophorogram reflected a common molecular weight of 22 kDa, 46 kDa, and 58 kDa when compared to the standard molecular weight marker of protein (broad range 11-75 kDa)

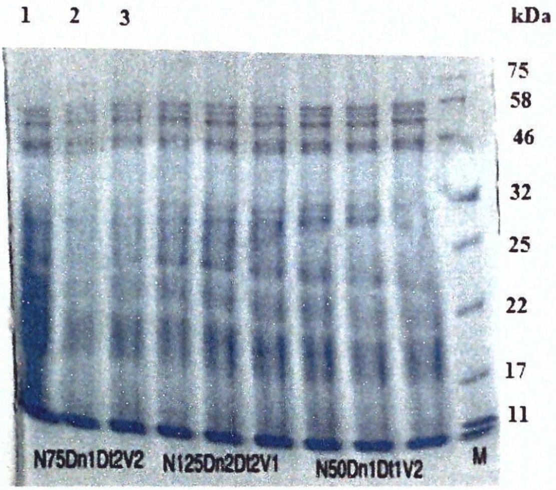

Figure 4.76: One dimensional SDS-PAGE separation of proteins of wheat genotypes 14SAWYT308 and Baviaans replicated 3 times. Only first 3 wells representing $N_{75}Dn_1Dt_2V_2$ were considered. Molecular weight maker was represented by label M.

In Figure 4.77 banding patterns of wheat proteins shown were from genotype Baviaans from 21st April at 333,333 plants/ha density with nitrogen at the rate of 125 kg/ha ($N_{125}Dn_1Dt_1V_2$), Genotype Baviaans planted on the 21st April at 250,000 plants/ha applied 75 kg/ha of N ($N_{75}Dn_2Dt_1V_2$), and Genotype Baviaans from 5th May planting date at 333,333 plants/ha with no nitrogen ($N_0Dn_1Dt_2V_2$). Their protein patterns were detected to be similar and were all under main cluster 3. Binary data matrix analysis showed that protein bands from electrophorogram had molecular weight of 25 kDa, 32 kDa, 58 kDa, and 75 kDa.

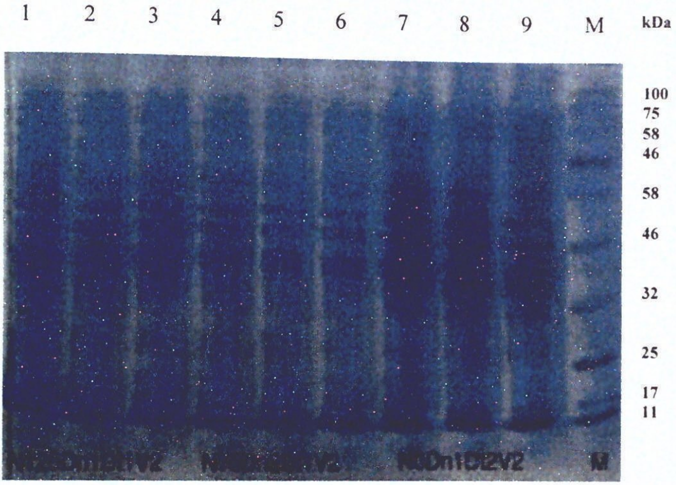

Figure 4.77: One dimensional SDS-PAGE separation of proteins of wheat genotype 14SAWYT308. Molecular weight maker was represented by label M.

In Figure 4.78 the treatment represented in the gel was from genotype 14SAWYT308 from first planting date at 333,333 plants/ha density with 0 kg N ha^{-1} ($N_0Dn_1Dt_1V_2$), which represented a sample treatment under main cluster 4. According to binary data matrix analysis present protein bands were of molecular weight 17kDa, 22 kDa, 32 kDa, 46 kDa, 58 kDa and 75 kDa.

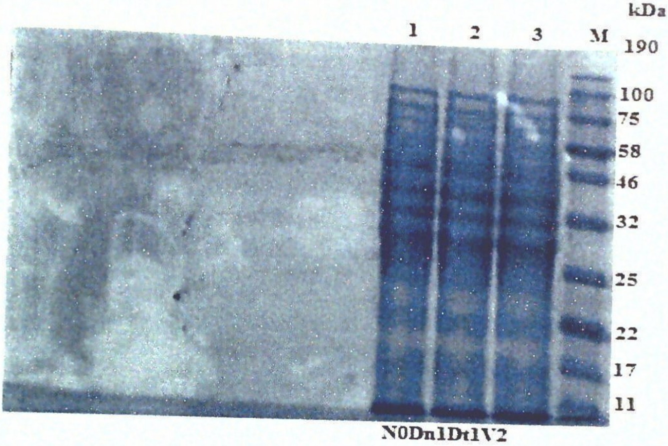

Figure 4.78: One dimensional SDS-PAGE separation of proteins of wheat genotype 14SAWYT308. Molecular weight maker was represented by label M.

Table 4.7 shows distribution of treatments sub-clusters based on protein bands. A total of 70 protein bands were detected, and were identified by common characteristics. The highest number of bands (17) were observed in sub-cluster F, defined by (all Baviaans genotype), represented by treatments $N_{125}Dn_1Dt_1V_2$, $N_{75}Dn_2Dt_1V_2$, and $N_0Dn_1Dt_2V_2$. Sub-clusters E and F contain 125 kg/ha N and account for 47% (33/70) of total protein bands, and the N_{125} in this experiment had higher protein content. Minimum number of protein bands (4) was obtained in sub-cluster C identified by all Baviaans genotypes from 21st April planting date ($N_0Dn_1Dt_1V_1$ and $N_{200}Dn_1Dt_1V_1$).

Table 4.7: Distribution of treatment sub-clusters based on protein bands characteristics.

Sub-cluster	**No. of protein bands**	**Cluster identification characteristics**	**Cluster members (Treatments)**
A	7	Genotype Baviaans planted at 250,000 plants/ha density	$N_{50}Dn_2Dt_2V_1$ $N_{75}Dn_2Dt_1V_1$
B	6	250,000 plants/ha density crops from 05th May planting date	$N_0Dn_2Dt_2V_1$ $N_{200}Dn_2Dt_2V_2$
C	4	Baviaans genotype from 21st April planting date	$N_0Dn_1Dt_1V_1$ $N_{200}Dn_2Dt_1V_1$
D	10	Crops at density of 333,333 plants/ha	$N_{200}Dn_1Dt_1V_2$ $N_{125}Dn_1Dt_2V_1$ $N_{125}Dn_1Dt_1V_1$
E	16	All crops from 21st April planting date	$N_{125}Dn_2Dt_1V_1$ $N_{200}Dn_2Dt_1V_2$ $N_{50}Dn_1Dt_1V_1$
F	17	All Baviaans genotype crops	$N_{125}Dn_1Dt_1V_2$ $N_{75}Dn_2Dt_1V_2$ $N_0Dn_1Dt_2V_2$
G	10	Genotype 14SAWYT308 planted at density of 333,333 plants/ha	$N_{50}Dn_1Dt_1V_2$ $N_0Dn_1Dt_1V_2$

CHAPTER FIVE

5.0 DISCUSSIONS

5.1 Effect of genotype on wheat yield and yield components

Wheat genotype 14SAWYT308 produced significantly more number of tillers, number of grains per spike, and 1000 grain mass than Baviaans (Table 4.2 and 4.3), and this might be due to its improved genetic characters (genetic superiority over Baviaans) and better adaptation to the prevailing environment (Munsif *et al.* 2015). Also these might have led to better utilisation of more photosynthates resources due to its larger leaf area to absorb more photosynthetically active radiation (PAR), hence accumulating significantly more grain (Figure 4.1 and 4.2). These is also explained by the genotype 14SAWYT308 having more biological, straw and grain yields than Baviaans (Table 4.3 and 4.4).

5.2 Effect of plant density on wheat yield components and yield parameters

In this study, all the yield components under study and plant height were significantly influenced by plant density, during both years. Significant differences existed in number of tillers, spike length, number of spikelets per spike, number of grains per spike and plant height as affected by plant density (Table 4.2a, b).

The 333,333 plants/ha density increased number of tillers by 24.29%, spike length (3.7%), number of spikelets per spike (6.74%), number of grains per spike (9.73%) and plant height (3.65%) compared to lower density of 250,000 plants/ha. The increase in yield components as density increases was attributed to higher values of leaf dry mass, crop growth rate and leaf area index (Figures 4.3, 4.5 and 4.6). Greater light was intercepted as a result of higher leaf area index, hence increased tiller development (Borger *et al.* 2010). The densely populated plants better utilised nutrients, and photosynthates for grain filling because of more absorption of photosynthetically active radiation due to larger leaf surface area, which resulted in increased yield components of wheat. The results are also consistent with those reported by Yadi *et al.* (2016), who reported that higher density increased number of spikelets per spike in wheat. The investigators reported highest number of spikelets (17.44) at maximum seed density compared to lower density which recorded 15.64 spikelets. In addition, Iqbal *et al.* (2012) reported a mean value 464.6 tillers/m^2 at higher plant population compared to 404.4 tillers/m^2 for lower population, while Baloch *et al.* (2010) recorded 311.8 tillers/m^2 at a higher seeding rate compared to 270.2 tillers/m^2 for lower seed density of wheat.

The tallest wheat plants were recorded in the 333,333 plants/ha treatment. The increase in plant height as density was attributed to increased light absorption (PAR) due to increased leaf area index (Figure 4.6) which led to more assimilates allocated to stem, hence increase in plant height (Lashkari *et al.* 2011). Yadi *et al.* (2016) reported similar results of increase in plant height with density when they obtained plant height of 109.2 cm at higher plant population. Other researchers, Naseri *et al.* (2012) reported taller plants with a mean value of 98.1 cm at higher plant density treatment of 400 plants/m^2 compared to lower density of 300 plants/m^2 (92.6 cm).

In terms of yield parameters, plant density significantly enhanced biological yield, harvest index (HI), and grain yield in both years, but straw yield was significantly influenced by plant density only in 2016 (Tables 4.3a and b). Increased straw yield at higher density is attributed to the higher number of tillers formed compared to the lower density of 250,000 plants/ha since tillers are a component of straw yield. These results agree with those of Ahmadi *et al.* (2011) who reported highest straw yield value of 13 310 kg/ha at maximum density of 500 plants/m^2. The higher biological yield recorded at higher density of 333,333 plants/ha was due to increased number of tillers, straw yield, number of spikelets per spike, and grain yield, since biological yield is the total above ground dry matter. The decrease in biological yield at lower density of 250,000 plants/ha was attributed to the decrease in light interception than at higher plant density as evidenced by lower leaf area index at Figure 4.6 (Amanullah *et al.* 2010). Due to fewer plants under lower density there was leaf surface to intercept PAR which is vital in the photosynthates manufacturing which normally results in more biological yield due to growth of the plant organs. Like with straw yield, studies have shown that biological yield increased with increasing plant density (Amanullah *et al.* 2010; Iqbal *et al.* 2012).

Harvest index denotes the ability of the plant to produce yield, and crops at 333,333 plants/ha density had a significantly greater harvest index, and was due to higher number of tillers, and number of spikelets per spikes, which resulted in higher grain yield, which is a factor of harvest index. These results are consistent with those of Naseri *et al.* (2012), who reported harvest index value of 48.6 with higher density of 400 plants/m^2 compared to lowest density of 300 plants/m^2 (43.8). Also supporting the results of this study, Iqbal *et al.* (2012) obtained harvest index value of 45.48 at higher seeding density compared to lower density which recorded 43.83.

During 2015 and 2016, the 333,333 plant density had significantly higher grain yield compared to 250,000 plants/ha. The grain yield of wheat was significantly increased by 11.2% and 15.8% at higher density for 2015 and 2016 seasons, respectively. In wheat, grain yield is influenced by yield components, and in this experiment the number of spikelets per spike and the number of grains per spike were significantly higher at higher density, which contributed to the increased grain yield. In addition, maximum grain yield is attributed to improvement in the number of tillers and the 1000 grain mass Tables (4.2a and b). In a favourable environment, there is a uniform yield due to regular tiller formation and to the distribution of photosynthates, which contribute to grain yield (Rickman *et al.* 1983). Improvement in ht interception during critical period for grain set may increase yields at higher densities ndrade *et al.* 2002). Therefore, at 333,333 plants/ha the plants had larger leaf area to tercept more light, which was a favourable growth condition that enabled better tillering, nce greater grain yield formation than at the lower density (Figure 4.6). Other studies uomo *et al.* 1998; Ferreira and Abreu, 2001) have demonstrated that increased grain eld with increasing plant density.

3 Effect of nitrogen application rate on wheat phenology, yield components, yield rameters and grain protein content

ddition of nitrogen fertiliser increased number of days to anthesis of wheat, and this was tributed to prolonged vegetative growth stage at tillering stage (28 DAS), as a result of creased leaf area index (Figure 4.12). However, nitrogen had no effect on number of days to nergence and physiological maturity of wheat.

this study nitrogen increased number of tillers by 31.73 % at 125 kg/ha of N compared to ntrol.The increased number of tillers at 125 kg/ha of N over control was attributed to creased vegetative growth as evidenced by more crop growth rate (figure 4.11) and leaf area dex (figures 4.12). This resulted in more allocation of assimilates to form more tillers at 125 g of N. Botella *et al.* (1993) attributed increased number of tillers at higher N to more imulation of tiller formation due to higher cytokinin to auxin ratio. Cytokinin metabolism and gnalling are closely related to nitrogen availability (Ruffele *et al.* 2011: Takei *et al.* 2004), nd nitrogen addition has been reported to elevate the cytokine levels in wheat (Garnia *et al.* 010). The results are in conformity with those Maqsood *et al.* (2014) who reported similar sults of nitrogen enhancing number of tillers when nitrogen was applied under semi-arid nvironment. They obtained maximum number of tillers/m^2 (362.87) at 120 kg/ha of N, ompared to 279.13 tillers/m^2 at 0 kg/ha of N treatment. Yousaf *et al.* (2014) also revealed maximum mean value of 402 tillers/m^2 when 150 kg/ha of nitrogen fertilizer was applied, compared to 92 tillers/m^2 with control treatment.

Application of nitrogen at 125 kg/ha also increased spike length, number of spikelets per spike (17.76%), number of grains per spike and 1000-grain mass (15.2%) over the control treatment. Increased N led to more vegetative grow (Figure 4.11), which increased number of spikelets per spike, spike length, and larger leaf area index (Figure 4.6) resulted in increased assimilates to produce more number of grains per spike and increased 1000-grain mass. At 125kg/ha of N, nitrogen nutrition was non-limiting and it enabled the crops to absorb more photosynthetically active radiation to manufacture more assimilates due to larger leaf area index (Figure 4.6), which channelled more assimilates towards grain formation (Fisher, 1985).

Results of the current study are in agreement with those of Maqsood *et al.* (2014) who observed wheat spike length of 10.25 cm at 120 kg/ha of N treatment compared to 9.84 cm spike length for the control treatment. Similar results of longer spikes when nitrogen was added compared to the control were also reported by Abdo *et al.* (2012) and Gerba *et al.* (2013).

Also in agreement with current results, Khan *et al.* (2000), noted an increase in the number of spikelets per spike at 120 kg/ha of N compared to the control treatment which had the least number of spikes. Iqbal *et al.* (2012) also reported a highest mean of 17.22 spikelets per spike at 125 kg/ha of N dosage compared to a low mean of 12.33 spikelets/spike under 0 kg/ha of N treatment when working on wheat.

Shahzad *et al.* (2013) reported highest number of grains/spike (80) when 120 kg/ha of N was applied, compared to 75 grains per spike in the control treatment. On a silty- loam soil, Yousaf *et al.* (2014) noted similar results of increased grain per spike with nitrogen as they recorded a maximum number of 47 grains under 120 kg/ha of N compared to 23 grains per spike in the control treatment. The 1000- grain mass results of this study are in agreement with those of other researchers working with various wheat varieties under similar conditions (Maqsood *et al.* 2014; Matusso *et al.* 2016 and Shahzad *et al.* 2013).

Over the two years, nitrogen application significantly affected plant height of wheat genotypes (Table 4.2a and 4.2b). The tallest plants were recorded at the nitrogen dose of 125 kg/ha, and means were significantly different between all nitrogen treatments. At the rate of 125 kg/ha, nitrogen was enough to increase the protein content of the cells, as a result cell size increased (evident by more crop growth rate reflected at figure 4.11), consequently the leaf area enlarged (figure 4.12), and photosynthetic activity increased and ultimately plant height increased too (Table 4.2a and b), Winsock *et al.* 2007. The results of increased plant height at higher N level are supported by Liaqat *et al.* (2003).

With reference to yield parameters, nitrogen application increased the straw yield of wheat genotypes during both years, with the highest straw mean yield recorded at a nitrogen dose of 125 kg/ha, which was significantly different from those of other nitrogen treatments (Table 4.3a, b). The increase in straw yield is attributed to more total leaf area (Figure 4.12). Similar results of an increase in straw yield with addition of nitrogen fertiliser were reported by Maqsood *et al.* (2014), who recorded highest mean straw yield mean of 9467.33 kg/ha at 180 kg/ha N over 6178.33 kg/ha for control. Haileselassie *et al.* (2014) under rain-fed conditions, obtained straw yield mean value of 5715 kg/ha at 138 kg/ha N, compared to straw yield mean value of 3897 kg/ha with the control treatment.

In this study, nitrogen fertiliser significantly enhanced both straw and grain yield of wheat in both years (Table 4.3a, b). The highest grain yield was recorded at a nitrogen dose of 125 kg/ha. The increased grain yield in plots that received N is attributed to the increased number of tillers, number of spikelets per spike and 1000- grain mass. Malhi *et al.* 2006 observed that nitrogen being the essential part of chlorophyll, enough was supplied to help in photosynthates manufacturing, resulting in suitable amount of nitrogen in plant tissues, hence increased yield. These results of current study are in accordance with those of Iqbal *et al.* (2012) who found that nitrogen at 125 kg/ha resulted in higher yield of 4629 kg/ha, compared to the control which recorded 3193 kg/ha. From the literature and this study, it is clear that similar dosage of N can produce different yields depending on prevailing environmental conditions. For instance, Yousaf *et al.* (2014) obtained 3100 kg/ha grain yield under 120 kg/ha of N treatment compared to 1690 kg/ha yield of control treatment, while Matusso *et al.* (2016), under rain-fed conditions, reported maximum grain yield of 1140 kg/ha under nitrogen dose of 46.8 kg/ha compared to 250 kg/ha yield for control treatment. In Serbia, Gorjanovic *et al.* (2010) obtained the highest grain yield of 8000 kg/ha with variety Malyska when supplied with 100 kg/ha of N compared to 5150 kg/ha grain yield of control treatment.

It is notable, however that 200 kg/ha of nitrogen reduced grain yield and most grain yield components of wheat compared to the rate of 125 kg/ha of N. On average for the two seasons, it was shown that the grain yield from 200 kg/ha of N was about 36% lower than of 125 kg/ha of N (Tables 4.3a and b). The crop growth rate (CGR) increased from the vegetative stage (28 DAS) when tillers where formed, up to heading stage (56 DAS) when 200 kg/ha of N was added (Figure 4.11). However when the plants reached flowering stage (84 DAS) there was less CGR suggesting that plant have used more assimilates for vegetation growth at the expense of grain filling. Decreased yield and yield components due to increased vegetative growth was reported by Gandapur and Bhatthi (1993). Yield increase magnitude may be less with continually increasing fertiliser application, with a plateau appearing at a certain threshold, indicating excessive N might limit the benefit (Dang *et al.* 2006). In addition reduced grain yield under the higher N is due to lighter grains, reduced number of spikelets per spike, and shorter spikes due to excess nitrogen (Tables 4.2 and 4.3). Brown and Petrie (2006) reported reduced wheat grain yield due to lighter kernels when nitrogen was added in excess above 100 kg/ha.

Addition of nitrogen fertilizer up to 125 kg/ha significantly increased biological yield of wheat during both seasons (Table 4.3a, b). The nitrogen application gave more number of tillers, straw yield, taller plants, and more grain yield and more total dry matter which collectively resulted in higher biological yield. Also nitrogen increased above ground matter by increasing leaf area index (Figure 4.12). which increased mainly due to increased number of tillers (Sylvester-Bradley, 1990). Therefore, an increase in biological yield (above ground dry matter) is as a result of increased total leaf area as the larger canopy is able to incept more PAR to produce more photosynthates. This results were supported by Ghobadi *et al.* (2010).

The results from this investigation show that nitrogen increased harvest index by 30.4% and 32.4% at 125 kg/ha of N compared to control for 2015 and 2016 respectively (Table 4.3a, b). When supplied with 125 kg/ha of nitrogen fertiliser, the crops were able to utilise it efficiently to form photosynthates and convert dry matter to grain yield, thus increased harvest index value. These results are in conformity with those of Shahzad *et al.* (2013) who obtained highest harvest index value of 29.0% when applying nitrogen fertiliser to wheat at rate of 120 kg/ha, compared to control treatment obtained 28.0 %. In addition, Maqsood *et al.* (2014) showed that addition of nitrogen increased harvest index of wheat crop as they reported harvest index value of 42.19% under 120 kg/ha of N treatment, compared to 32.97% harvest index value at control.

The application of N at a rate of 125 kg/ha significantly increased protein content relative to other treatments for both seasons (Table 4.4). Increased grain protein content with nitrogen increase is attributed to greater availability of nitrogen at grain filling stage as guided by more leaf dry mass at grain filling stage (112 DAS) (Figure 4.10). These findings were substantiated by Alam (2012) who reported a grain protein content of 12.99% when wheat crop received 160 kg/ha of N compared to 11.26% of grain protein under control treatment. Similar results of increase in grain protein as nitrogen was increased was reported by Nakano *et al.* (2008) and Mattas *et al.* (2011). Reduced grain protein content at 200 kg/ha of N dose are due to excess nitrogen which caused the crops to grow more vegetatively at the expense of grain filling, resulting in some plants lodging as shown by reduced plant height at 200 kg/ha (Table 4.3a and b), hence reduced grain protein accumulation in the grains. Similar findings were reported by Abedi *et al.* (2011) who observed a significant increase in grain protein content with N rates of 120 and 240 kg/ha, but reduced grain protein content at 360 kg/ha of N while working on wheat. Also supporting the results of the current study, Campillo *et al.* (2010) reported increased grain protein content when nitrogen was added from 0, 150, 200, and 250 kg/ha, thereafter the grain protein content of the wheat decreased when applying 300 kg/ha of N during the 2005/2006 planting season.

5.4 Effect of planting date on wheat phenology, yield components, yield parameters and grain protein content

The wheat crops planted on 05th May took longer to emerge than those planted on 21st April during both seasons. Delayed emergence in the 5th May planting may be due to slightly lower average minimum temperature (8.43 ^{O}C and 9.6 ^{O}C for 2015 and 2016 respectively), compared to those of April (14.6 ^{O}C and 15.6 ^{O}C) for 2015 and 2016, respectively (Appendix 2). The lower temperature in the month of May have slowed down germination and ultimately emergence. Normally when temperatures go down the process of germination becomes slow and ultimately the crown root initiation stage is prolonged, resulting in delayed emergence. Earlier research has demonstrated that growth of later-planted wheat is generally slower because of lower temperatures (Joshi *et al.* 1992; Qasim *et al.* 2008), and this might have contributed to significantly longer days to emergence by seedlings planted on the 05th of May. Analysis of data presented on Table 4.1a and 4.1b indicated that the number of days to anthesis was significantly affected by planting date. The 21st April plants took more time to reach anthesis than those planted on 05th May. This is probably as a result of lower temperatures in month of July coinciding with anthesis period (min. 5.6 °C and 4.2 °C for 2015 and 2016 respectively) for the 21st April sowing date which probably lowered photosynthetic activity which resulted in delayed anthesis. In a study by Sud and Arora, (1990), earlier sown crops had vigorous vegetative growth, which took longer time, and as a result anthesis was delayed. In this experiment, planting date did not have a significant effect on crop growth rate, although the CGR value was maximum at flowering (Figure 4.17). It is therefore unlikely that vigorous vegetative growth might be the cause for delayed anthesis, hence the lower temperature effect is probable as explained above.

Earlier planted wheat crops (21st April) took significantly longer time to reach physiological maturity compared to later planted crops (5th May). Probable reason for delayed maturity in earlier planting is due to lower temperature in August (8.4- 27.9 °C and 6.8 – 26.2 °C for 2015 and 2016, respectively) for the 21st April date coinciding with last stage of grain filing duration which was slowed down hence resulting in longer days to maturity compared to the September month coinciding with grain filing for 5th May plants. The results agree with findings of Sial *et al.* (2005), who concluded that longer maturity period was taken by early planted crops. In addition, Sandhu *et al.* (1999) reported similar findings of longer physiological maturity with earlier planting due to effect of lower temperature and more grain filling time.

With reference to yield components, data from Table 4.2a and 4.2b revealed that sowing date significantly affected the number of tillers per unit area in both years. Planting date of 21st April produced significantly higher number of tillers, compared to the 05th May date. Thus, earlier planting produced more tillers due to higher temperature which promoted tillering (Table 4.9). In 2015, average minimum and maximum temperatures in May was 8.4 °C and 27.8 °C respectively, and coincided with tillering of the (21st April) earlier planted crops, that is, it was warmer than the June temperature (min 4.8 °C and max 21.8 °C) which coincided with (5th May) later planted crops. Number of tillers per plant at a given time may be reduced by low temperatures due to slower leaf development on the main axis resulting in reduction of maximum possible tillers (Assuero and Tagnetti, 2010). Similar results of increased number of tillers at earlier date of planting compared to later planting were recorded by other investigators (Baloch *et al.* 2012; Shah *et al.* 2006).

The longest spikes were recorded during the first planting date, while shorter spikes were noted in the later planting time in both years, and the means were significantly different. The increased spike length in earlier planting is attributed to better spike development due to a longer growing period (Farooq *et al.* 2016). El-Gizawy (2009) also obtained similar results of reduced spike length in the later sowing with a minimum length of 10.0 cm compared to maximum length of 10.9 cm at earlier planting. Also Baloch *et al.* (2012) reported longer spikes (11.04 cm) under first planting treatment against shorter spikes (10.91 cm) from later planting date. Shah *et al.* (2006) also found a highest mean value of spike length (10.0 cm) when planting wheat at an earlier date than later planting where they recorded 9.58 cm spike length under similar conditions.

Planting date significantly affected the number of grains per spike of wheat during the 2015 and 2016, respectively. Significantly higher number of grains per spike and number of spikelets/spike were obtained from plants sown on 21st April compared to the 05th May planting date. More grain in earlier date is due to more production of photosynthates as a result of longer growing period (Tahir *et al.* 2009), hence more grain. Mumtaz *et al.* (2015) reported similar results of decrease in grains per spike as planting date was delayed, as they obtained minimum value of 54.00 grains in the first planting treatment against maximum of 55.83 grains under the second planting. These results are also supported by findings of Shahzad *et al.* (2002) who conveyed higher number of grains/spike (51.00) during first planting compared to second planting (49.25).

Planting date significantly enhanced the 1000- grain mass and the number of spikelets per spike of wheat genotypes in both years. Earlier sown wheat plants produced significantly higher 1000- grain mass and number of spikelets per spike relative to that of later planting. Later planting resulted in lesser number of spikelets per spike and poor development of grains due to shorter growing period which resulted in less partitioning to the reproductive sink resulting in lower 1000- grain mass (Gul, 2012; Tahir *et al.* 2009; Baloch *et al.* 2012; Eslami *et al.* 2014; Muntaz *et al.* 2015). In addition the decrease in number of spikelets per spike with delayed planting date might be due to a reduction in spike length, although in some cases spikelets per spike are small as a characteristic of a variety (Hussain, 1995).

Taller plants were observed during the 21st April planting time, compared to 05th May planting date during both years, and the differences in means were significantly different (Table 4.2a and 4.b). The earlier planted crops have had longer vegetative growth period than later planted crops, which resulted in more cell elongation hence increased plant height (Quasim *et al.* 2008). Baloch *et al.* (2012) reported similar results of decrease in plant height due to late sowing in wheat when they obtained minimum plant height of 90.33cm under later date of 10th November compared to an earlier date of 20th October (105.9 cm). Likewise, El-Giwazy (2009) and Munsif *et al.* (2015) obtained similar results as those of this study.

Regarding yield related parameters, in both years planting date had a significant effect on straw yield, with highest straw yield recorded during the earlier planting date (Table 4.3). The higher straw yield at early planting was a result of more tillers and taller plants which increased biological yield, which is a factor in straw yield determination (Amrawat *et al.* 2013; El-Gizawy 2009; Tahir *et al.* 2009).

Planting date had a significant effect on grain yield of wheat for the two years with significantly more grain yield obtained on the 21st April planting, compared to 05th May planting. Earlier planting increased grain yield by 23.6% and 22.9% for 2015 and 2016 respectively, compared to later planting. The increase in grain yield with earlier planting is due to the increase in yield attributes like number of grains/spike, number of tillers and 1000- grain mass. This is also due to the increased number of tillers produced at earlier planting, more filled spikes and plump seed which resulted in more grain yield (Said *et al.* 2012). This results of increased grain yield at earlier planting date are supported by Khokhar *et al.* 2010, Mahboob *et al.* 2005, Munsif *et al.* 2015, Mumtaz *et al.* 2015 and Shahzad *et al.* 2007.

Earlier planting of 21st April resulted in 16.6% (2015) and 15.7% (2016) increased biological yield compared to 05th May planting (Table 4.3a and 4.3b). Since biological yield comprised of above ground dry matter, in this case number of tillers, number of spikelets per spikes and straw yield were higher in earlier planting, it was inevitable that the biological yield of 21st April was higher than that of later planting. Increased biological yield in earlier planting compared to later planting results in this current study agreed with those of Amrawat *et al.* 2013, Shah *et al.* 2006 and Wajid, 2004.

The highest harvest index was recorded at 21st April planting date, and this increase suggests that temperature conditions were optimum (warmer) to transform photosynthetic matter from source (vegetative organs) to sink (grain). Lower mean value of harvest index was recorded at 05th May planting date, indicating that transformation of photosynthetic matter to grain was done hardly due to lack of suitable producing yield components in vegetative growth period. The results are in conformity with those of Amrawat *et al.* (2013) who recorded maximum harvest index value of 37.30% under earlier planting compared to later date (35.84%). El-Gizawy (2009) shared similar sentiments when the researcher obtained higher harvest index value of 39.4% at earlier planting treatment compared to 38.6% for later planting.

The higher protein content (GPC) was recorded during the 21st April planting, with lower protein content recorded at later planting date. Grain filling period was longer at the earlier sowing, and more assimilates, together with vegetative N was channelled to grains resulting in improved grain protein content. Yan *et al.* (2008) reported that the highest grain protein content and grain yield can be produced by planting wheat crop at proper sowing time. Therefore it is probable that the 21st April date under this study provided a conducive environment for the wheat crop to express the higher GPC at earlier planting. The results of increased GPC with earlier planting are in agreement with those of El-Gizawy (2009) and Eslami *et al.* (2014).

5.5 Relations between grain yield and yield components

The relationship between grain yield and yield components of wheat is showed a positive significant relationship as shown in Table 4.5. The highest significant correlation was between grain yield and number of tillers (r=0.72), while the lowest correlation was between spike length and 1000- grain mass, and was non-significant (r=0.12). This implies that the number of tillers was the highest contributing factor to grain yield of wheat under this study. In wheat tillers bear spikes and spikelets which ultimately enclose grain, therefore increased number of tillers may result in more grain yield. Yield components, for instance, number of spikelets per spike, number of grains per spike and 1000- grain mass also contributed significantly to formation of grain yield. Okumura *et al.* (2011) and Cruz *et al.* (2008) reported similar findings of positive correlation between yield and yield traits as observed between number of tillers, spike length, number of spikelets per spike, number of grains per spike, 1000- grain mass and grain yield.

5.6 Effect of significant interactions on some traits of wheat

5.6.1 Effect of plant density and planting date interaction on some wheat traits

In this study interaction of plant density and planting date significantly increased number of tillers, number of spikelets per spike, number of grains per spike, biological yield and grain yield of wheat during 2015 and 2016 (Figure 4.21 – 4.30). The highest values were recorded at 333,333 plants/ha at the 21st April planting date, significantly different from interaction of 250,000 plants/ha at 05th May planting date. There was more leaf area which enabled more light interception and better tillering, resulting in more yield formation (Figure 4.6).

5.6.2 Effect of plant density and nitrogen application rate interaction on some wheat traits

The interaction of plant density and nitrogen significantly influenced the number of tillers, number of spikelets per spike, number of grains per spike, biological yield and grain yield (Figure 4.31- 4.39). Crops at 333,333 plants/ha with 125 kg/ha of nitrogen gave highest mean values of number of tillers, number of spikelets per spike, number of grains per spike, biological yield and grain yield, significantly different from that of crops at 250,000 plants/ha and control nitrogen treatment. Increased yield and yield components at higher density and 125 kg/ha of N is attributed to increased number of tillers, number of spikelets per spike and 1000-grain mass as a result of increased leaf area index, hence more assimilates accumulation (Figure 4.12.

5.6.3 Effect of plant density, planting date and genotype interaction on some wheat traits

The highest number of tillers, number of grains per spike, biological yield and grain yield were recorded from genotype 14SAWYT308, planted on 21st April planting date at a density of 333,333 plants/ha and the mean was significantly different from that of genotype 14SAWYT308 under same planting date but different density of 250,000 plants/ha (Figure 4.40 – 4.46). However, higher number of spikelets per spike was highest at Baviaans planted on 21st April, at 333,333 plants/ha. The higher yield and yield components for genotype 14SAWYT308 grown on first planting at higher density is a result of more production of photosynthates as a result of longer growing period for the earlier planting date (Tahir *et al.* 2009).

5.6.4 Effect of nitrogen application rate and planting date interaction on some wheat traits

The combination of 125 kg/ha of N and 21st April planting date led to higher yield and yield components except for number of spikelets per spike (Figure 4.39-4.46). Increased yield and yield components at 21st April planting date with 125 kg/ha of N is attributed to most suitable climatic conditions during 21st April date, where crops were able to utilise nitrogen efficiently to form photosynthates to increase yield and yield components (Said *et al.* 2012).

5.6.5 Interaction of nitrogen application rate, plant density and genotype effect on some wheat traits

Genotype 14SAWYT308 fertilised with 125 kg/ha of nitrogen at 333,333 plants/ha density produced highest mean biological yield and number of spikelets per spike (Figure 4.55-4.63). Crops which received a dose of 125 kg/ha of nitrogen, under higher density increased number of tillers due to more stimulation of tiller formation as a result of higher cytokinin to auxin ratio as nitrogen was added (Botella *et al.* 1993). This could have led to the crops having better utilised enough moisture, adequate nitrogen, and photosynthates for improvement of yield components and improved yield.

5.6.6 Interaction of planting date, plant density and nitrogen application rate with respect to some wheat traits

The highest values for number of tillers, number of grains per spike and grain yield was obtained from crops of 21st April planting date, at density of 333,333 plants/ha with nitrogen rate of 125 kg/ha added to them (Figure 4.64-4.73). The mean significantly differed with means obtained from crops at 05th May planting date, 333,333 plants/ha density, and nitrogen dose of 125 kg/ha. Increased yield components and yield at first planting, higher density, and 125 kg/ha nitrogen dose is attributed to increased number of spikelet per spikes, one thousand grain mass and number of grains per spike, which contributed to the increased grain yield. Improvement in light interception during critical period for grain set also increased yields at higher densities (Andrade *et al.* 2002). In addition 125 kg/ha of nitrogen nutrition was enough, resulting in the crops absorbing more photosynthetically active radiation to synthesise more assimilates, which were channelled towards grain formation and increased yield and yield components (Fisher 1985). Akbar *et al.* (2002) reported increased grain yield on the interaction of nitrogen rate and density.

5.7 Protein profiling of wheat

Glutenins are said to be divided into two major groups, the High Molecular Weight (HMW: 70 – 120 kDa), and Low Molecular Weight (LMW: 35 – 50 kDa), according to the electrophoretic mobility in Sodium Dodecyl Sulphate – Polyacrylamide Gel Electrophoresis (SDS – PAGE) (Pfluger *et. al.* 2001; Gianibelli *et al.* 2001).The two wheat genotypes were clustered in a dendrogram showing protein band size similarities based on the 40 treatments of the current study using Un-weighted Pair Group Method with Arithmetic mean cluster analysis (UPGMA) (Figure 4.66).

From the four main clusters demarcated at coefficient of ≈ 0.63, the highest number of treatment interactions was recorded in main cluster 3. The main cluster 3 was represented by genotype Baviaans from 21st April planting date at 333,333 plants/ha that received nitrogen at rate of 125 kg/ha ($N_{125}Dn_1Dt_1V_2$), genotype Baviaans from 21st April planting date at 250,000 plants/ha with 75 kg/ha of N ($N_{75}Dn_2Dt_1V_2$), and Baviaans from the 05th May planting date at 333,333 plants/ha with nil nitrogen fertilizer ($N_0Dn_1Dt_2V_2$) (Figure 4.69). Protein bands identified were of sizes 25, 32, 58, and 75 kDa, and these results suggest the presence of LMW and HMW glutenin in the cluster 3 treatments (Pfluger *et al.* 2001). The sub-clusters of main cluster 3 were grouped into A to F, on which demarcation of treatments was at coefficient 0.88 or more, implying that they share at least 88 % HMW and/or LMW protein bands amongst them.

The main cluster 2 comprised of only one treatment interaction (Figure 4.68), which showed genotype 14SAWYT308 from 05th May at density of 333,333 plants/ha and 75 kg/ha of N fertilizer ($N_{75}Dn_1Dt_2V_2$). The binary data matrix analysis of this cluster was able to identify protein band sizes of 22, 46, and 58 kDa, thus, classifying them mainly as low molecular weight glutenin.

The main cluster 1 representative (Figure 4.67) of genotype 14SAWYT308 from 05th May planting date at density of 333,333 plants/ha and 50 kg/ha of nitrogen fertilizer ($N_{50}Dn_1Dt_2V_2$) showed that the treatment interaction contained protein of band sizes 11 to 58 kDa, suggesting that they are low molecular weight protein glutennins (Khan *et al.* 2006). Low Molecular Weight glutenins have been documented to contain Glu-A3d, Glu-B3b and Glu-B3g subunits, which are associated with good baking quality (Branlard *et al.* 2003: Liang *et al.* 2010).

The main cluster 4 was represented by genotype 14SAWYT308 from 21st April planting date at 333,333 plants/ha density with nil nitrogen ($N_0Dn_1Dt_1V_2$) (Figure 4.70). Proteins detected on them were of sizes 17, 22, 32, 46, 58, and 75 kDa, suggesting presence of both HMW glutenin (MacRichie and Lafiandra 2001) and LMW glutenins. Normally all the HMW and LMW sub-units are bound together in glutenin polymers, the size and composition are strongly associated with glutein quality (Lew *et al.* 1992). High molecular weight glutenin subunits (HMW-GS) as components of glutenin polymer also play a main role in the determination of the unique viscoelastic properties of wheat dough (Taladriz *et al.* 1994).This is because they are known to contain Glu-D1 genes, which has effect on the rheological properties and baking quality of flour (Luo *et al.* 2001).

Protein quality is the result of the combination of genotype and environmental factors (Mariani *et al.* 1995).The results from Table 4.7 showed that a total of 70 protein bands identified by common characteristics were detected and distributed among the 40 treatments of the study. The highest number of bands (17) were observed in sub-cluster F, characterised by (all Baviaans genotypes), represented by treatments $N_{125}Dn_1Dt_1V_2$, $N_{75}Dn_2Dt_1V_2$, and $N_0Dn_1Dt_2V_2$. Sub cluster E also contained equally many protein bands (16) as the sub cluster E, and together they account for 47% of the total protein bands (33/70). Both of these sub-clusters contain the 125 kg of N which contributed most to the protein bands. In wheat flour baking quality normally increase with protein level, so the dominance of 125 kg/ha N in the clustering of protein bands may indicate good baking quality for such cluster members. Therefore, the effect of genotype (all Baviaans at sub-cluster F) and nitrogen (dominance of 125 kg/ha of N at both cluster E and F), and 21st April planting date (16 bands from sub-cluster E) seem to have played a bigger role in clustering of protein bands rather than plant population (Table 4.7).

CHAPTER SIX

6.0 CONCLUSIONS

Plant density of 333,333 plants/ha gave higher grain yield and enhanced most yield components than 250,000 plants/ha density. Plant density of 333,333 plants/ha increased grain yield of wheat by 11.2% and 13.6% over the density of 250,000 plants/ha for 2015 and 2016 seasons, respectively. Results from the study suggests that wheat genotypes 14SAWYT308 and Baviaans should be planted at denser population density of 333,333 plants/ha to improve yield (0.15m * 0.2m).

Nitrogen input had positive effects on grain yield and yield components of wheat as it improved them. Grain yield increased up to 125 kg/ha of N, thereafter did not increase yield further. Grain yield was increased by 64.0% (2015) and 66.1% (2016) at 125 kg/ha of nitrogen rate over the control. Planting date of 21st April produced more grain yield (23.6% in 2015 and 22.9% in 2016) compared to 5th May date over the two years. Genotype 14SAWYT308 exhibited better yield and yield components characters than Baviaans when planted early. The best performing treatment was planting genotype 14SAWYT308 at density of 333,333 plants/ha on 21st April. It was found that wheat genotypes 14SAWYT308 and Baviaans produced maximum yield when planted on the 21st April at 333,333 plants/ha and nitrogen application rate of 125 kg/ha to maximize yield under South Eastern part of Botswana.

Grain from studied treatments showed the presence of proteins with molecular weight ranging from 17 kDa to 75 kDa. Protein bands with a molecular weight of 22 kDa, 32 kDa, and 58 kDa were identified to be most contributors to variability in treatments clustering. The results showed that the presence of glutenins of both high molecular weight and low molecular weight are likely to contain genes that supports good baking quality of flour, and also indicate a considerable amount of genetic diversity between studied treatments. Therefore based on protein profiling the interaction of plant population, planting time and N at 125 kg/ha contributed more protein bands with low and high molecular weight glutenins and that would produce wheat flour with good baking qualities. Grain protein bands seem to have been influenced mainly by addition of N, genotype and planting date rather than population.

CHAPTER SEVEN

7.0 RECOMMENDATIONS

The two wheat genotypes are suitable for growing under the Botswana conditions in the Southern part of the country. More trials should be carried out in other agro-ecological zones of the country. Pertaining to protein profiling, gels should be analysed at two dimensional SDS-PAGE to generate more data on variability of treatments. Farmers should grow wheat in Botswana under irrigation.

CHAPTER EIGHT

8.0 REFERENCES

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CHAPTER NINE

9.1 APPENDIX 1

WHEAT FIELD PLAN 2015 and 2016 SEASONS

BLOCK 1		BLOCK 2		BLOCK 3	
$N_{75}Dn_2Dt_2V_1$	plot 140	$N_{125}Dn_1Dt_1V_2$	plot 240	$N_{75}Dn_2Dt_1V_1$	plot 340
$N_0Dn_1Dt_1V_1$		$N_{75}Dn_2Dt_2V_2$		$N_0Dn_2Dt_1V_1$	
$N_{200}Dn_2Dt_2V_1$		$N_0Dn_1Dt_1V_2$		$N_{200}Dn_1Dt_2V_1$	
$N_{50}Dn_1Dt_1V_1$		$N_{200}Dn_1Dt_1V_2$		$N_{50}Dn_1Dt_2V_1$	
$N_{200}Dn_2Dt_1V_1$		$N_{75}Dn_1Dt_2V_2$		$N_{125}Dn_1Dt_1V_1$	
$N_0Dn_1Dt_2V_1$		$N_0Dn_2Dt_2V_2$		$N_{75}Dn_2Dt_2V_1$	
$N_{75}Dn_1Dt_2V_1$		$N_{50}Dn_2Dt_2V_2$		$N_0Dn_2Dt_2V_1$	
$N_{125}Dn_2Dt_1V_1$		$N_{75}Dn_2Dt_1V_2$		$N_{75}Dn_1Dt_1V_1$	
$N_{50}Dn_2Dt_1V_1$		$N_{200}Dn_2Dt_2V_2$		$N_{125}Dn_2Dt_2V_1$	
$N_0Dn_2Dt_2V_1$		$N_{125}Dn_2Dt_2V_2$		$N_{50}Dn_2Dt_2V_1$	
$N_{200}Dn_1Dt_2V_1$		$N_0Dn_2Dt_1V_2$		$N_{200}Dn_1Dt_1V_1$	
$N_{75}Dn_1Dt_1V_1$		$N_{200}Dn_2Dt_1V_2$		$N_{125}Dn_1Dt_2V_1$	
$N_{125}Dn_1Dt_2V_1$		$N_0Dn_1Dt_2V_2$		$N_{200}Dn_2Dt_2V_1$	
$N_{200}Dn_1Dt_1V_1$		$N_{75}Dn_1Dt_1V_2$		$N_{50}Dn_1Dt_1V_1$	
$N_0Dn_2Dt_1V_1$		$N_{50}Dn_1Dt_2V_2$		$N_0Dn_1Dt_1V_1$	
$N_{50}Dn_1Dt_2V_1$		$N_{125}Dn_2Dt_1V_2$		$N_0Dn_1Dt_2V_1$	
$N_{125}Dn_1Dt_1V_1$		$N_{125}Dn_1Dt_2V_2$		$N_{75}Dn_1Dt_2V_1$	
$N_{125}Dn_2Dt_2V_1$		$N_{50}Dn_2Dt_1V_2$		$N_{125}Dn_2Dt_1V_1$	
$N_{75}Dn_2Dt_1V_1$		$N_{50}Dn_1Dt_1V_2$		$N_{50}Dn_2Dt_1V_1$	
$N_{50}Dn_2Dt_2V_1$	plot 121	$N_{200}Dn_1Dt_2V_2$	plot 221	$N_{200}Dn_2Dt_1V_1$	plot 321
$N_0Dn_1Dt_1V_2$		$N_{125}Dn_1Dt_2V_1$		$N_{125}Dn_1Dt_2V_2$	
$N_{75}Dn_1Dt_1V_2$		$N_0Dn_2Dt_1V_1$		$N_{50}Dn_2Dt_1V_2$	
$N_{200}Dn_2Dt_1V_2$		$N_{50}Dn_2Dt_2V_1$		$N_0Dn_2Dt_1V_2$	
$N_{50}Dn_2Dt_1V_2$		$N_0Dn_1Dt_1V_1$		$N_{75}Dn_2Dt_2V_2$	
$N_{75}Dn_2Dt_2V_2$		$N_{125}Dn_1Dt_1V_1$		$N_{200}Dn_2Dt_1V_2$	
$N_{75}Dn_2Dt_1V_2$		$N_{75}Dn_2Dt_2V_1$		$N_{50}Dn_1Dt_2V_2$	
$N_0Dn_2Dt_2V_2$		$N_{200}Dn_1Dt_2V_1$		$N_{200}Dn_2Dt_2V_2$	
$N_{200}Dn_1Dt_2V_2$		$N_{200}Dn_2Dt_1V_1$		$N_0Dn_1Dt_1V_2$	
$N_{200}Dn_1Dt_1V_2$		$N_{50}Dn_1Dt_2V_1$		$N_{75}Dn_2Dt_1V_2$	
$N_{50}Dn_1Dt_1V_2$		$N_{200}Dn_1Dt_1V_1$		$N_{50}Dn_2Dt_2V_2$	
$N_{125}Dn_2Dt_2V_2$		$N_{75}Dn_1Dt_1V_1$		$N_{200}Dn_1Dt_2V_2$	
$N_{125}Dn_1Dt_1V_2$		$N_0Dn_2Dt_2V_1$		$N_{50}Dn_1Dt_1V_2$	
$N_{200}Dn_2Dt_2V_2$		$N_{75}Dn_2Dt_1V_1$		$N_{75}Dn_1Dt_2V_2$	
$N_{50}Dn_1Dt_2V_2$		$N_{200}Dn_2Dt_2V_1$		$N_0Dn_2Dt_2V_2$	
$N_0Dn_2Dt_1V_2$		$N_{50}Dn_1Dt_1V_1$		$N_{125}Dn_1Dt_1V_2$	
$N_{125}Dn_1Dt_2V_2$		$N_{125}Dn_2Dt_2V_1$		$N_{200}Dn_1Dt_1V_2$	
$N_{50}Dn_2Dt_2V_2$		$N_0Dn_1Dt_2V_1$		$N_0Dn_1Dt_2V_2$	
$N_{75}Dn_1Dt_2V_2$		$N_{75}Dn_1Dt_2V_1$		$N_{125}Dn_2Dt_1V_2$	
$N_{125}Dn_2Dt_1V_2$		$N_{125}Dn_2Dt_1V_1$		$N_{125}Dn_2Dt_2V_2$	
$N_0Dn_1Dt_2V_2$	plot 101	$N_{50}Dn_2Dt_1V_1$	plot 201	$N_{75}Dn_1Dt_1V_2$	plot 301

N= nitrogen rate (0, 50, 75, 125, 200 kg/ha) **D_n= plant density 0.15m*0.2m (333,333 plants/ha), 0.2m*0.2m (250,000 plants/ha)**

Dt_1= planting date 21st April Dt_2 planting date 05th May V_1=Baviaans, V_2=14SAWYT308

9.2 Appendix 2

Meteorological data for Sebele from April 2015 to December 2016

Month April 2015- December 2016	Temperature (°C)				Relative Humidity (%)	Solar Radiation (w/mm)	Sunshine (hours)	Rainfall the growing months (mm)
	Average min	Extreme min	Average max	Extreme max				
April-2015	14.6	11.6	27.86	32.3	61	41	7.8	12.3
May	8.43	5.2	27.87	32.1	42	43	9.9	6.1
June	4.78	-1.3	21.88	25.8	47	33	9.4	5.3
July	5.67	-1.1	22.88	28.6	43	36	9.8	6.8
August	8.42	-1.9	27.97	33.6	32	41	9.6	8.6
September	14.07	6.8	28.85	37.2	40	44	9.1	10.4
October	18.64	10.2	34.55	38.9	28	58	10.6	-
November	18.11	13.6	33.45	41.5	30	60	10.5	-
December	22.55	17.9	35.54	40.2	37	59	10.1	-
January-2016	21.65	17.3	33.79	42.7	52	45	9.1	-
February	21.98	16.6	34.65	39.6	61	36	8.9	-
March	18.06	10.6	30.27	34.2	62	36	8.2	-
April	15.6	12.7	28.92	33.8	59	30	8.7	8.9
May	9.65	3.4	24.16	28.4	60	27	9.7	8.4
June	6.15	2.6	22.20	26.2	62	25	9.6	3.2
July	4.15	-2.3	22.25	28.3	46	29	9.4	3.8
August	6.84	1.6	26.22	30.9	32	33	9.5	4.2
September	13.96	7.2	30.94	35.9	29	37	9.0	7.3
October	18.25	8.5	33.54	38.6	33	44	9.6	-
November	19.83	15.8	32.74	37.2	38	44	10.4	-
December	20.91	17.8	32.07	37.9	-	-	10.0	-

(Adopted from Department of Meteorological Services, 2016)

9.3 Appendix 3

9.3 Solutions for Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) (Laemmli, 1970).

Solution A (3.0 M Tris-HCL pH 9.0, 0.4 % SDS)

- Tris 36.3 g
- SDS 0.4 g
- Distilled water + concentrated HCL 70mL and adjusted to pH 8.8
- Made total volume of 100 mL

Solution B (0.49 M Tris-HCL pH 7.0, 0.4 % SDS)

- Tris 5.98 g
- SDS 0.4 g
- Distilled water 80 mL and adjust pH 7.0
- Make total volume of 100 mL

Solution C (30 % Acrylamide, Acrylamide/Bis= (30:0:8)

- Acrylamide 30 g
- Bis-acrylamide (Bis) 0.8 g
- Distilled water and made to volume of 100 mL

Staining solution

- Methanol 440 mL
- Acetic acid 60 mL
- Distilled water 500 mL
- Coomassie Brilliant Blue (CBB) R250 2.25 g
- Made up to total volume of 1 litre

10 % APS

- Ammonium per sulphate (APS) 0.1 g
- Distilled water made to total volume of 1 mL

Destaining solution

- Acetic acid 50 mL
- Methanol 200 mL
- Distilled water 750 mL

Protein Extraction Buffer (0.05M Tris-HCL Ph 8.0, 0.2 % SDS, 5 M Urea, 1 % β-mecaptoethanol)

SDS	0.2 g
Tris	0.6057 g
Urea	30.3 g
Distilled water + concentrated HCL	70 ml adjusted to pH 8.0
β-mecaptoethanol	
Made to total volume of 100 ml by adding distilled water	

Electrode buffer solution (0.025 M Tris, 0.129 M Glycine, 0.125 % SDS)

Tris	3.0 g
Glycine	14.4 g
Distilled water to a total volume of 1 litre	

Gels solutions (1 mm thick)

Separation Gel (12.25 %)

Solution A	5 mL
Solution C	7.5 mL
TEMED 15	µL
10 % APS	200 µL
Distilled water	7.5 mL
Total volume of 20 mL	

Stacking Gel (4.5 %)

Solution B	2.5 mL
Solution C	1.5 mL
10 % APS	70 µL
TEMED	17 µL
Distilled water	6.0 mL
Total volume 10 mL	