Evaluation of Nitrogen Use Efficiency (NUE) in Wheat

By

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DECLARATION

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Bahaddin Abdullah Faraj and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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List of Abbreviations

ADP	Di-ammonium phosphate
AGT	Australian Grain Technology
ANOVA	Analysis of variance
GH	Glasshouse
GY	Grain Yield
HI	Harvest Index
LSD	Least significant difference
N	Nitrogen
NH_4^+	Ammonium
NO_x	Nitrous oxide
NO_3^-	Nitrate
NUE	Nitrogen Use Efficiency
NupE	Nitrogen uptake efficiency
NutE	Nitrogen utilization efficiency
TGN	Total grain nitrogen content
TGW	Thousand grain weight

Abstract

Nitrogen fertilizers are a major input required for cereal crop production worldwide. The management of this resource is a significant challenge to most agricultural systems as it can have significant impacts on yield and the environment. The use of applied nitrogen fertilisers in cereals is poor, where only 30-40% is actually used by cereals and the remainder lost to the environment by surface runoff, soil denitrification and volatilization (Ehdaie et al., 2010; Butterbach-Bahl and Dannenmann 2011). Improving cereal nitrogen use efficiency (NUE) is imperative to achieve yield and quality with less direct N inputs. In this study, experiments were conducted in 2010 to evaluate the effect of N fertilizer application (0, 50, 100, 150 kg urea/ha) on the growth and yield of wheat varieties at specific locations across South Australia while a small pilot glasshouse study was conducted at the Waite Campus, Adelaide University. The field experiments were designed as a randomised split-plot with three replications for each wheat cultivar and N treatment. Plant response to N treatment was measured through estimates of plant height, leaf chlorophyll content (SPAD meter), plant spike number, grain yield, 1000 grain weight, shoot biomass weight, grain N % and final grain protein content, harvest index (HI) and NUE. Restrictions in space and large growing pots limited the controlled glasshouse study to a technical study.

The results found little variability between the three field sites in Grain %N in response to increasing N provision. There was a trend of increasing grain %N at both Mintaro and Pinnaroo, which was broadly in evidence across the individual lines. Grain yield was highest at Mintaro and was double of that achieved at both Pinnaroo and Tuckey. Whereas, in the glasshouse experimental results show that there was a strong response in grain %N to increasing N provision when plants were grown over the spring/summer season but not during the autumn/winter. Nitrogen use efficiency (NUE) was found to be greater at low nitrogen treatment (N1) in all experiments and decreased roughly with increased N application. In general, the results indicated that wheat cultivars responded well to nitrogen application with the medium rate of application within experiments, while beyond this rate caused no significant improvements in plant growth and yield.

Key words: Wheat varieties, nitrogen fertiliser (varied levels), nitrogen use efficiency (NUE), Yield, grain protein content

CHAPTER 1

Introductory Background

Nitrogen (N) is the most limiting nutrient required for food productivity worldwide (Giller et al., 2004). Over the past four decades, the doubling of global agricultural food production has been reached in part with a 7-fold increase in the use of N fertilizers, where approximately 90-100 million metric tonnes are used for agricultural production (London et al., 2005). Global population growth has led to a significant increase in demand of cereal crops and other agriculture products. World population growth is expected to reach 8 billion people by 2025 further increasing the demand for food and greater efficiency in productivity. However, a great challenge will be to do this in an environmentally sustainable manner. One direction which will have an influence is the development of novel plant genotypes which have a greater capacity to produce harvestable yields using less external inputs such as nitrogen fertilisers. These genotypes should have the capacity to accumulate and or assimilate N more efficiently than that of previously selected crops while still maintaining the required harvested production levels demanded of farmers and consumers (Hirel et al., 2007).

Nitrogen can only be used by plants in its reduced form. Unfortunately, the majority of N in the environment is in the form of di-nitrogen (N_2) which comprises ~ 72% v/v of the air on the planet. Available forms of N (e.g. NH_3 & NO_3^-) can occur through the activity of lightning, biological nitrogen fixation and via the energy intensive Haber-Bosch process. Plants such as legumes can form an effective N_2-fixing symbiosis with soil bacteria, where they obtain the necessary levels of N from the atmosphere to adequately balance the demands required for growth and successful seed production. However in non-legume crops, N must be acquired in a reduced form where demand can vary widely depending on the targeted yield and final protein content of the harvested product. Furthermore, differences in plant genotypes, environmental interactions and management systems will influence the supply and demand by the plant for N (Angus et al., 2001).

The majority of non N_2-fixing agricultural crops are dependent on introduced N-fertilizers to grow and set seed. In developed countries, fertilizers mainly consist of N, which is often poorly represented in agricultural soils. N-fertilisers are therefore heavily relied upon to improve crop growth and to deliver sufficient food to supply both animal and human needs. The extensive use of N fertiliser unfortunately has an impact on the local environment

through changes in soil microbe activity and non-agricultural plant and animal ecosystems. In most situations, about 50% to 75% of nitrogen fertiliser applied to agricultural crops is not used by the intended crop but rather is lost to the environment through leaching, volatilisation or indirectly through the activity and competition of soil microorganisms. Moreover, the capacity of plants to capture nitrogen from soil can be dependent on the soil type, environmental conditions (warm versus cold) and plant species. Consequently, nitrogen fertilizers are the largest source of N released into the atmosphere worldwide, which also represents a significant amount of the greenhouse gas emissions (nitrous oxide (NO_x) and the emission of toxic ammonia into the atmosphere (Ramos et al., 1996; Stulen et al., 1998). N in particular has direct effects on water quality where N fertiliser release (NO_3^-) from agricultural soils is often linked to eutrophication of freshwater and/or marine ecosystems (Beman et al., 2005). When applied in excess, high nitrate content in plant tissues can cause toxicity problems (methemoglobinemia) in humans especially in infant children and also in drinking water contaminated with high NO_3^-, and livestock from grazing on contaminated grass (Bruning-Fan &Kaneene 1993). Preventing excessive nitrate accumulation in crop species is important to avoid potential community health problems. With these ongoing issues it is important that agricultural crop plants are developed with greater NUE to effectively capture and assimilate N to maximize growth per unit of applied N fertiliser. Improvements in NUE will not only help to minimize production costs in crops as in wheat and corn (up to 40%) (Bock et al., 1984) through less fertiliser applications but also decrease the environmental damages associated with N-fertiliser application (Good et al., 2007).

The objectives of this study were to provide a better understanding of wheat varieties (genotypes) and their responses to different levels of N-fertilisation under controlled (glasshouse) and uncontrolled (field) conditions and to identify genotypic differences in NUE traits across an array of wheat lines. These experiments are likely to lead to improvements in both grain yield and corresponding grain protein content and at the same time minimize economic losses and environmental risks while maximizing grower's production capacity and their income.

CHAPTER 2

2. Review of Literature

2.1 Introduction

This literature analysis focuses on research that has characterized the relationship between nitrogen (N)-fertilization and the production of wheat. As part of the review, plant N use will be discussed and the issues associated with N-fertilizer use in agriculture and the directions for identifying and improving N use efficiency (NUE).

2.2 Wheat is a global agricultural crop

Wheat is a major world food crop grown in both developed and developing countries. It sits in relative importance to that of maize and rice as essential crops required to meet present and future food demands (Joshi et al., 2007a). Of these crops, wheat represents approximately 30% of current total cereal production (Fageria et al., 1997). In 2009-2010, global wheat production was approximately 645 million tons (Quail et al., 2011). It is expected that this value will need to increase significantly to match food demands of a growing world population by 2050. How this will be achieved is unknown, as simple expansion of cultivated arable land is not expected to increase but rather decrease as a result of growing urbanization, increased soil erosion and drought and salinity affected areas (Joshi et al., 2007). Technical advancements will thus be important to improve genetic resources that provide agronomists and breeders the necessary tools to increase future wheat production on a predicted smaller agricultural footprint. Improvements associated with wheat production may include: 1) the development and selection of new genotypes that can be grown productively using lower inputs, 2) the use of compatible modern agricultural techniques which retain soil health and improve nutrient and water delivery and efficiencies, and 3) germplasm development which improves resistance to biotic and abiotic stresses. One direction, which will provide significant improvement to productivity and agricultural sustainability, is the efficient management of applied nutrients. N in particular has been an agricultural input, which has generated much interest due to its rising cost of production; a process that is dependent on non-renewable resources. Furthermore, N fertilizers can have a negative impact on the environment when application is not efficiently met by plant utilization. Improving N use efficiency in agriculture is a global aspiration and is the basis of this work.

Impact of Australian dryland wheat production on N use

Overall, Australian dryland-farming systems are using farm enterprise diversity (especially mixed crop–livestock systems) with pastures and fallow periods to maximize the efficient use of available resources (fertilizer inputs and limited water). Moisture is usually the deciding factor in the success of cereal cropping alongside fertilizer inputs. Moreover, weather has a major influence on crop growth and grain yield (N demand), and on the availability of soil and fertilizer-N (N supply). The difficulty of predicting the weather more than a few days ahead is a major barrier to making accurate fertilizer recommendations. Plants in natural field conditions face changing environmental conditions where N concentrations vary and frequently are limiting for growth due to many factors including surface run-off, leaching of rain-water, soil erosion, and gaseous losses by volatilization and consumption by soil microorganisms. Moreover, N nutrition management is difficult because its effect on plant growth, development and physiology is related to unpredictable soil moisture under rainfed conditions (Basso et al., 2010). Adequate N fertilization is necessary to produce high yields of wheat and to increase grain quality (protein content). High levels of protein are important for superior wheat flour and baking characteristics (Feil et al., 1997). However, the environmental and genetic interactions controlling seed protein content are extensive (Kramer et al., 1979) and need to be defined in relation to favourable and unfavourable growing conditions.

2.3 Role of N in plant growth

N is an important factor limiting plant growth and productivity worldwide. Plants are provided N from both atmospheric air and soil minerals. The ability of plants to capture N naturally or that applied as N fertilizers, is one of the critical steps limiting the efficient use of N by plants. Despite N being one of the most abundant elements on earth, N deficiency is one of the most common problems affecting plant growth worldwide. Plants lacking N show stunted growth and yellowish leaves and will often fail to meet expected yields.

Plants in general contain 3-5% N in their shoot tissue biomass, which is by far the most abundant soil derived nutrient outside of oxygen, hydrogen and carbon. Plants absorb N from the soil in the form of NO_3^- and NH_4^+ ions. Most N uptake is in the form of NO_3^-, which moves from the soil solution into the plant root cell with absorbed water. NO_3^- is then either stored in the vacuole or reduced in the cytosol and plastids eventually to NH_4^+ through the

activity of nitrate and nitrite reductase (NR, NiR) respectively. NH_4^+ can then be assimilated to produce more complex N containing compounds (Lam et al., 1996). These compounds include chlorophyll that captures light to be used during photosynthesis. N is also an essential component of nucleic acids (DNA and RNA), amino acids, vitamins (e.g.: biotin, thiamine, niacin and riboflavin) and all proteins and a vast array of N containing organic molecules.

N uptake by plant roots

The uptake of N-fertilizers occurs when N is readily available in the soil solution at the root zone and when plant demand for N exists. When these conditions are met, NO_3^- and NH_4^+ transporter systems (see below) are expressed across various cell types across the roots for initial uptake and redistribution across the root to the stele (Daniel-Vedele et al., 1998; Tsay et al., 2007). The form of N ion taken up by plants depends on the plant species and soil properties including texture and chemistry. Plants that grow in low pH anaerobic soils, NH_4^+ or amino acids are often the preferred N form. In contrast NO_3^- uptake is more prevalent in aerobic and neutral pH soils (Maathuis et al., 2009). N uptake by wheat has shown preference between N forms, where net uptake was increased 35% when supplying at least 25% of the N as NH_4^+ compared to all of the N as NO_3^- (Wang and Below 1992). It is assumed this selectivity is partly a result of the energy costs required for NO_3^- assimilation relative to NH_4^+. Assimilation of NO_3^- requires the energy equivalent of 20 ATP per mol of NO_3^-, whereas NH_4^+ assimilation requires only 5 ATP mol^{-1} of NH_4^+ (Salsac et al., 1987). This energy savings could lead to more efficient N and C capture when plants are supplied with greater amounts of NH_4^+ (Huffman et al., 1989).

N is a mobile element in the soil where its uptake from the soil solution is best met when plant demand exists. The quantity and rate of N uptake is very much dependent on the ion concentration in the soil solution, the availability of water which supplies the majority of N to the roots through mass flow and the capacity of the roots (position and density) to enable plant interception (Russell et al., 1977). Mass flow is a process where dissolved N ions in soil water is delivered to the root based on the hydraulic pull of water to the plant as a result of shoot evapotranspiration (Barber et al., 1984). The concentration of N in the soil solution close to the root may be high or low depending on the balance between the rate of supply to the root and the rate of absorption into the root. N can also diffuse to the root following a concentration gradient from high to low, however the rate of diffusion is governed by distance, thus diffusion only really becomes important close to the root surface. Root

interception is influenced by root density and surface area, which varies due to soil structure, plant genetics and agronomy practice. Maximum root surface area enables greater capacity for ion absorption from the soil solution and or soil particles (Barber et al., 1984).

N has a strong influence on root development in most plants. Plants tend to develop smaller roots when N is readily available in the soil solution. Under these conditions it is the plant's physiological capacity to accumulate and assimilate N through the activity of N transport and assimilatory proteins rather than through changes in root morphology (Glass et al., 2003). At low levels of N in the soil solution, many plants will commit extra carbon resources to further develop root systems to enable greater penetration of the soil (Garnett et al., 2009). However, increased carbon delivery to the root will have consequences on shoot biomass and potentially yield penalties. Therefore, from a NUE context, traits that enhance N acquisition (efficiency and activity) without increased demand on plant carbon to roots will be a favourable direction in any NUE breeding or trait selection program (Fageria &Baligar 2005; Hirel et al., 2007, Garnett et al., 2009).

Improving the ability of root systems to recover soil NO_3^- by earlier and faster uptake is an efficient strategy to improve the synchronization between the availability of NO_3^- in the soil profile and the NO_3^- demand by the wheat crop (Liao et al., 2004). This strategy states that roots grow fast in order to intercept and capture the NO_3^- before it moves below the rooting profile. Genotypic differences associated early root biomass, root branching and root length have been linked to the early uptake of N in wheat genotypes that differ in overall vigour (Liao et al., 2004; 2006). When N uptake occurs, both the xylem and phloem is involved in transporting N in the plant. The xylem is the principle path for long distance transport of N solutes (NO_3^- and NH_4^+) from the roots to organs that transpire (Pate et al., 1980). The xylem therefore transports NO_3^- from the roots to shoots in addition to N reduced to NH_4^+ in the roots (Schrader et al., 1984). The phloem is the principal transport path of N stored or assimilated in the shoot and transported to other parts of the plant (i.e. leaf to seed) (Liao et al., 2006).

Nitrogen transport proteins

NO_3^- and NH_4^+ are predominant forms of inorganic N, and their movement from the soil into root cells is dependent on at least two transport systems for each ion (Forde et al., 2000; Howitt and Udvardi 2000). In Arabidopsis, the total influx of NO_3^- into the root is a result of

a combination of four additive influxes: constitutive high-affinity influx (cHATS), inducible high-affinity influx (iHATS), constitutive low-affinity influx (cLATS), and inducible low-affinity influx (iLATS) (Forde et al., 2000). High-affinity activities occur at low concentrations of ions (10-250 µM) and low affinity activities occur at high concentrations of ions > 250 µM. The uptake of NO_3^- occurs across the plasma membrane of epidermal and cortical root cells via NO_3^- transporters encoded by two multigene families (NRT1 and NRT2) (Glass and Crawford 1998). The NRT1 family relates to low-affinity transporters (LATS) and belongs to the peptide transporter family (PTR), which consists of 53 genes in the model plant Arabidopsis thaliana, 13 of which are experimentally proven to be functional transporters of NO_3^- (Tsay et al., 2007). The NRT2 family relates to high-affinity NO_3^- transporters (HATS). In Arabidopsis, seven NRT2 genes have been identified (Tsay et al., 2007) and are preferentially expressed in roots. In tomato, no LeNRT2 expression is observed in whole shoots or leaves (Ono et al., 2000) while in tobacco (Nicotiana plumbaginifolia), NpNRT2 transcripts are also detected at low levels in leaves, petioles, buds, flowers, and seeds (Quesada et al., 1997). In Arabidopsis, RT-PCR using specific primers for AtNRT2.1 and AtNRT2.2 revealed that the expression of NRT2.1 and NRT2.2 in young seedlings appears 10 days after sowing, but is not detectable at earlier stages (Zhuo et al., 1999). These experiments indicate that in Arabidopsis NRT2.1, NRT2.2 and NRT2.4, although expressed at different levels, show similar patterns of root expression. In contrast, it seems that NRT2.7 is preferentially expressed in shoots, suggesting a role in leaf NO_3^- uptake.

The uptake of NH_4^+ involves the transporter family AMT, which belongs to a larger group of NH_4^+ transporters of the ammonium/methyl ammonium/Rhesus (AMT/Mep/Rh) family (von Wiren & Merrick 2004). Heterologous expression of selected plant AMTs in (Xenopus oocytes) indicate that they function as NH_4^+ uniporters that transport NH_4^+ along an electrochemical gradient (Ludewig et al., 2003). In Arabidopsis, six ATM genes have been identified including (AtAMT1; 1 – AtAMT1; 5) and four genes in tomato and ten genes in rice have been identified (Loque &von Wiren, 2004). In Arabidopsis, AtAMT1; 1 and AtAMT1; 3 have been localized to the plasma membrane of epidermal and cortical root cells and have shown to be responsible for 30% of the NH_4^+ uptake capacity (Kaiser et al., 2000; Loque et al., 2006). Recent work has shown that AtAMT1;4 is predominantly expressed in pollen and interestingly capable of enhancing NH_4^+ uptake when over expressed in roots of a AMT deficient mutant (qko) (Yuan et al., 2007).

Nitrogen assimilatory enzymes

Nitrate is the predominant form of inorganic N in agricultural soils and is therefore used by many plant species. NO_3^- enters root cells where it is either stored in the vacuole or reduced and incorporated into amino acids. N assimilatory enzymes which are linked to this N uptake process in roots include nitrate reductase (NR); nitrite reductase (NiR) and the glutamine synthetase (GS) / glutamine-2-oxo-glutarate aminotransferase (GOGAT) pathway. NO_3^- that enters the cell can be reduced by NR to NO_2^-. NO_2^- is toxic in the cell cytosol and is rapidly transported into the plastid for further reduction to ammonium by NiR. Generated ammonium then enters the glutamine synthetase (GS) and glutamate synthesis (GOGAT) cycle where it is converted into glutamine and glutamate (Oaks et al., 1994; Lam et al., 1996). Ammonium present in the cytosol of the plant cells can also be assimilated directly to glutamine via GS activity and then used in a series of transamination reactions to produce amino acids. Moreover, NH_4^+ is constantly being produced in leaf mitochondria during the photorespiratory N cycle. This NH_4^+ pool is re-assimilated by glutamine synthesis (GS) in the chloroplast or by Glutamate Dehydrogenase (GDH - is the primary route for the assimilation of ammonia in plants) activity in the mitochondria (Cuturier et al., 2007). These reactions are regulated by the GS/GOGAT pathway active in the chloroplast or cytosol of plant cells. In leaves, these interactions are at the expense of primary products of photosynthesis and compete with the reduction of carbon. In roots, stored or translocated carbohydrates serve as the primary substrate for carbon and energy requirement of N assimilation. Therefore, N assimilation seems to be different in roots and shoots (Oaks & Hirel, 1985). A hypothesis that GDH plays an important role in controlling glutamate homeostasis has been put forward. This function, which may have a signalling role at the interface of C and N metabolism, may be of importance under certain phases of plant growth and development when there is an important release or accumulation of NH_3 (Terce-Laforgue et al., 2004). Moreover, the major catalytic activity for GDH in plant cells has been reported to be glutamate de-amination (Masclaux-Daubresse et al., 2006; Purnell and Botella, 2007) and GDH activity was shown to be essential for plant survival in dark conditions (Miyashita and Good 2008).

Nitrogen is mostly taken up during the vegetative phase of wheat phenological development. N applied early in the season stimulates tillering and vegetative plant growth, while N applied late in the season has a greater influence on the final N concentration in the grain (Fajersson

et al., 1961; Lütke Entrup & Oemichen 2000). Higher N use efficiency can be found when N is applied late (at flowering) than it is applied early in the season (Raun & Johnson 1999; Cassman et al 2002). N fertilizer rates and types and application times influence N indices such as N uptake (Iqbal et al., 2005) and its translocation within the plant (Kichey et al., 2007). Thus fertilizer application timing has a significant role in determining the quantity of uptake and its use by the plant (Limaux et al., 1999). N in aboveground plant parts is actively recycled and transported to the grain as the plants mature (Simpson et al., 1983; Cooper et al., 1986). This redistribution of N from aboveground plant parts to grain has been broadly studied (Austin et al., 1977; Van Sanford & MacKown 1987; Feller & Fischer 1994; Fangmeier et al., 1999; Masclaux et al., 2001). The flow and amount of N redistributed to developing seeds will vary depending on the source-sink ratio, which is regulated by the weather (temperature, drought) and the inherent properties of the organs (Dalling et al., 1976). It has also been suggested, remobilisation of N from the roots may play an important role in the final N economics of the whole plant (Dalling, Boland & Wilson 1976; Simpson, Lambers & Dalling 1983). Roots have been suggested to play a major role in assimilating N when the crop is suffering N deficiencies in the shoot (Vouillot & Devienne-Barret 1999) that is followed on by remobilizing N to the grain during grain filling.

Relationship to carbon metabolism

N assimilation is closely linked to carbon metabolism through the need of carbon skeletons required for amino acid synthesis and energy equivalents (ATP and reductant) to power many of the N assimilatory genes (Martin et al., 2002). A plants capacity to fix CO_2 is highly dependent on its N status as all of the proteins involved in light capture and fixation of CO_2 contain large amounts of N. Rubisco alone consists of approximately ~ 50% of plants total protein content. When N is deficient, these enzymes are often the first to show the affects of N limitation, namely increased chlorosis. Sugars produced through photosynthetic CO_2 assimilation are either stored (starch and or sugar) or respired. The breakdown of these sugar molecules is important as they provide the building blocks for amino acid biosynthesis. Thus all developmental processes from germination through flowering and seed development will be strongly influenced by the balance between available C and N and how these two elements can work together (Rolland et al., 2002). N is important for the assimilation of carbohydrates in the plant and plays an important role in root growth for the absorption of other essential minerals including K and P.

Other uses of N (i.e. NO_3^-)

Nitrate also has an important role as a signaling molecule in plant cells where its presence can initiate the synthesis of both nitrate transport and assimilating enzymes (Crawford et al, 1995). Moreover, NO_3^- has an effect on carbohydrate metabolism where it influences the relationship between both starch and sucrose synthesis and NO_3^- can also act as a counter ion in the uptake and reduction of malate (Samuelson et al., 1995).

2.4 What is N Use Efficiency?

N use efficiency (NUE) of a crop plant refers to the relative balance between the amount of fertilizer taken up and then used by the crop versus the amount of fertilizer supplied directly or indirectly (Nielsen et al., 2006). In other words, NUE looks at fertilizer input recovery in a production system to classify which plants do this better or worse when compared equally based on production (yield). NUE is defined by many authors in the context of crop production and the literature contains a number of different definitions depending on whether authors are dealing with agronomic, genetic, or physiological studies (Good et al., 2004; Fageria et al., 2008). Moll et al., 1982, first defined NUE as grain production per unit of available N in the soil. This definition included two components; 1) N uptake efficiency (N in the plant per unit of N fertilizer applied), and 2) N utilization efficiency (grain yield per unit of N in the plant). A similar measurement was developed by (Semenov et al., 2007) who defined NUE as the ratio between yield and the input of N minerals regardless of source (NUE = Y/Ns), where Ns (kg/ha) is available N for the plant during the growth period, including initial inorganic N in the soil, applied N fertilizer, and mineralized N from organic N uptake during the growth period, and Y is grain yield (kg/ha). There are also alternative definitions of NUE in the literature (Cassman et al., 1998; Tilman et al., 2002; Raun et al., 2002) which along with the previous mentioned definitions are accompanied by considerable disagreement of which NUE definition is best used and / or which is appropriate.

Moll et al., (1982) considered that N uptake efficiency (NupE) is the primary component determining NUE when soil N supply increases. This is explained by N uptake exceeding the critical value of N content in crop dry matter (Lawlor et al., 2001, Lemaire and Millard 1999). On the other hand, Ortiz-Monasterio et al., (1997) reported that N uptake is also an important component of NUE under low N conditions. Both (Ortiz-Monasterio et al., 1997; and Le Gouis et al., 2000) stated that NupE in wheat accounts for most of the variation in

NUE at low N availability. Ortiz-Monasterio et al., (1997) further defined NupE to include harvest index (HI) and biomass production efficiency (BPE) affirming that HI is best associated with NupE. Reductions in NUE as N supply increases could result from reductions in any of the components, including NupE, NutE and N retention efficiency (NRE). Studies on wheat and perennial grasses have shown various limitations in each of these components (Cox et al., 1985; Dhugga and Waines 1989; Huggins and Pan 2003; Jiang et al., 2000; Morris and Paulsen 1985; Oritz-Monasterio et al., 1997). For example, Oritz-Monasterio et al., (1997) found that in all wheat varieties evaluated, both NupE and NutE were reduced at higher N supplies, causing an overall reduction in NUE. Morris and Paulsen (1985) and Cox et al., (1985) showed a reduction in N-translocation efficiency at high versus low N supply (Dhugga and Waines 1989).

NUE can be partitioned into the individual components of NupE and NutE (Moll et al., 1982; Oritz-Monasterio et al., 1997). NupE can be calculated as the total above-ground N per unit of N supplied, including available N from soil or not. Therefore, organic matter N mineralization plays an important role in the calculation of N uptake from the soil (Le Gouis et al., 2000). However, Youngquist et al., (1992) suggested that when initial soil N contents are equal, genotypic differences in NupE can be determined by measuring only plant N.

(Feil et al., 1992) indicated that cultivars producing large amounts of biomass seemed to have a more efficient nutrient uptake, which could decrease the total NUE of modern cultivars. Since N concentration is higher in leaves than in stems and sheaths, N uptake may be more closely related to leafiness than to total shoot biomass (Feil et al., 1997). Moreover, genetic differences in N recovery in the grain were mostly attributed to the net N uptake after anthesis rather than of remobilized N (Suprayogi et al., 2011). Post anthesis N uptake was found to be exponentially related to grain mass (Pan et al., 2006) but may vary with environmental conditions, such as N and water availability (Baresel et al, 2008).

For this work we have used the definition of NUE proposed by (Moll et. al., 1982). We have found this definition, which includes NupE and NutE, particularly useful in looking at genetic differences in NUE among wheat cultivars grown at both low and high input N regimes. This provides a framework for evaluating variation in N use among genotypes as related to major physiological processes.

2.5 Strategies to Improve NUE

Cereals require N-fertilizers to produce maximum yields and high protein content (Ortiz - Monasterio et al., 1997; Barraclough et al., 2010). However, NUE in cereals is generally poor, where it is estimated 30-40% of the total of N-fertilizers applied is actually harvested in the grain. The reminder of the applied N is lost to the soil, where often-excessive application can affect natural ecosystems through N pollution. Loss of N also contributes to significant direct economic losses to the grower particularly when N fertilizer costs are high (Raun and Johanson 1999; Glass et al., 2003; Gruber and Gilloway 2008). It has been estimated that an increase in NUE by one percent is worth as much as USD $234 million (Magen and Nosov 2008). Therefore, initiatives to improve NUE will be important in order to minimize both N-fertilizer losses and the direct production costs of the crop. On the basis of field experiments, (Cassman et al., 2002) reported N recovery in wheat varied from as low as 18 percent under unfavourable weather to 49 percent under favourable weather conditions. One of the main causes of low NUE in actual N management practices is the limited synchrony between N soil availability and crop demand (Raun and Johnson 1999; Cassmann et al., 2002; Fageria and Baligar 2005). Consequently, many different agronomic avenues are pursued to improve NUE in cereal crops which includes: 1) Application of the correct dose of N-fertilizer and/or application during growth stages when N is required; 2) Directed delivery of N to minimize losses or maximize utilization, for example, banding or point placement close to the root; 3) Use of cover crops, to retain organic matter and soil N in the soil; 4) Increased use of crop rotations (shallow and deep rooted crops), such as wheat following legumes, and avoiding wheat- fallow or wheat-wheat scenarios; 5) Use of modern farming techniques such as conservation tillage to control weed, soil moisture, erosion, operation costs and environment; 6) Identifying the best sowing rate, spacing and depth for best use of soil water and fertilizers and 7) The selection of wheat germplasm that produce larger seeds to ensure quick plant establishment and access to available N at the young seedling stage. Alongside any improvement in NUE related agronomy comparable improvements to plant germplasm must also occur. NUE traditionally hasn't been a central driver in genetic improvement programs that are driven by traditional breeding programs or marker based approaches including the use of quantitative trait loci (QTL) analysis (Quarrie et al., 2005). Furthermore there is a need to better understand N use in wheat, particularly the plant's capacity for mining N from the soil and its efficient use once within the plant.

2.6 Plant and soil factors influencing NUE

As mentioned previously, cereal NUE can be as low as 30-40% due to a range of biotic and agronomic-based factors. These include the primary growing conditions that influence overall photosynthesis and plant respiration such as day/night temperatures (Yoshida et al., 1982) and the amount and timing of precipitation (Kravcheckov et al., 2003). High-yielding varieties will often demand larger amounts of N fertilizer to meet expected yields or to improve grain quality (higher protein content). While pest and disease pressure will often affect demand for N, this can consequently reduce yield and NUE. Furthermore, the type of plant also has a dramatic impact on NUE. In general, cereal crops have higher N recovery efficiency (RE_N) than root crops, which in turn have a higher RE_N than leafy vegetables (Balasubramanian et al., 2004).

The impact of N fertilization on crop plants is very much influenced by the cycling of N between inorganic and organic forms and the relationship between the N present in the air, water and soil fractions. This transition of N activity is referred to as the N cycle, which describes the different forms and stages that N exists in the air, soil, water and the biological continuum. N is never lost completely in the cycle, but merely changes its form and availability (Mosier et al., 2004 and Smil et al., 1999) (Figure 2.1). The predominant changes include: (1) *Ammonification* which is the process where organic forms of N are converted to ammonium (NH_4^+). Soil organisms (bacteria and fungi) carry out the majority of ammonification. The organisms receive carbon, N and energy from the breakdown of organic matter, while excess N is released; (2) *Nitrification* is the process involving the conversion of NH_4^+ to nitrite (NO_2^-) and then to nitrate (NO_3^-). Soil organisms involved in nitrification processes get energy from the chemical transformation of NH_4^+ to NO_2^-; (3) *Denitrification* is the process where NO_3^- and NO_2^- are converted into gaseous N (NO_2, N_2) by microorganisms. Denitrification occurs mainly when there is little or no oxygen in the soil (e.g: soil is waterlogged). However, denitrification process stops when soil dries; (4) *N_2 fixation* is the conversion of N gas (N_2) to NH_4^+, either by free living bacteria in soil or water, or by bacteria in symbiotic association with plants (e.g: legume symbiosis); (5) *N immobilization* is the process whereby N is incorporated into microbial cells and effectively ‘tied-up’ in the 'microbial pool' of N. Immobilisation occurs in parallel with ammonification.

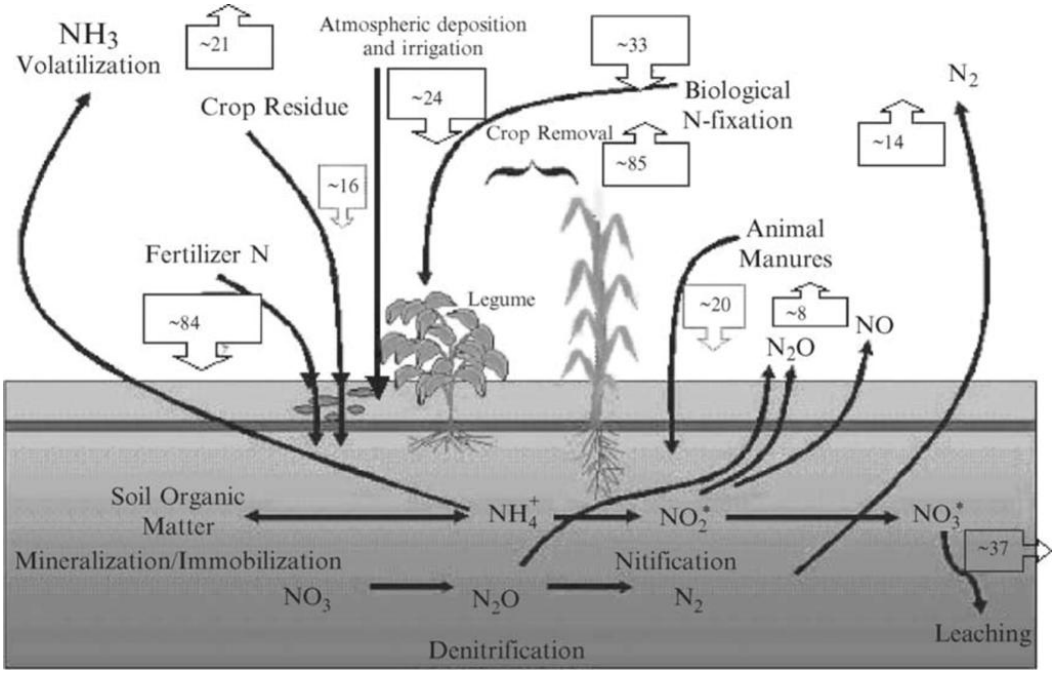

Figure 2.1: The global N balance in crop production (adapted from Mosier et al., (2004), and Smil et al., (1999). The figures are in Tg (10^{12} g) per year. Leaching (37 Tg) includes runoff and erosion losses; ammonia volatilization (21 Tg) includes volatilization from soil and vegetation.

Thus N cycling has a significant impact on the quantity and supply of N to the plant. A significant component of the N cycle involves soil-based microbial activity. This process is strongly influenced by the availability of organic C in the soil, which is used as a primary microbial energy source (Stevenson et al., 1994). Application of organic material or crop residues with high C: N ratios to the soil can stimulate microbial N immobilization, a process where available NH_4^+ and NO_3^- is competitively used by microbes. This process can reduce crop yield unless N is supplemented with applied fertilizers (Van Lauwe et al., 2002). Soil based constraints can also promote or decrease microbial based N cycling activities including denitrification, ammonia volatilization (Mosier et al., 2001a; Schlesinger et al., 1997). Excess water in poorly drained soils results in anaerobic conditions, which directly affects the rate of denitrification by nitrifying bacteria. This promotes an accumulation of NH_4^+ in the soil solution (van Kessel et al., 1993). The rate of volatilization of fertilizer N is then largely controlled by the pH and NH_4^+ content of the soil (Vlek and Craswell 1981).

2.7 Managing N use

Nitrogen is a dynamic and highly mobile element in agricultural soils causing environmental problems through increased N pollution that acts both locally and globally (Glass et al., 2003; Gruber and Galloway 2008). The extensive use of N-fertilizers in agriculture has created major problems worldwide through N based pollution of surface and underground water supplies. Therefore, concentrations of NO_3^- in agricultural products and drinking water should be minimized. Although the fact that the main source of NO_3^- intake is food, not water, the World Health Organization (WHO, 1970, modified in 1993) set a recommended limit for drinking water of 50 mg NO3- per litre. The main issue was the microbial conversion of NO_3^- to nitrite (NO_2^-), which was associated with problems involving nitrosamines and methaemoglobin. The so-called "blue-baby syndrome" (methaemoglobinaemia), for example, arises from bacteria-contamination and not from ingesting too much NO_3^- as originally supposed. Recent work even suggests that ingested NO_3^- provides gastro-intestinal protection against food-borne pathogens and "epidemiological studies show a reduced rate of gastric and intestinal cancer in groups with a high vegetable based nitrate intake" (Leifert and Golden 1997). Elevated concentrations of nitrate in streams or aquifers are mostly due to excessive or poorly used N applications in agriculture. High NO_3^- concentrations in water also occurs in years following drought. High NO_3^- concentrations in forage can cause sickness and death in livestock when grazing due to NO_3^- accumulation in plant tissue. The accumulation occurs due to high temperature, drought, other nutrients deficiency and plant disease (IFA, 2007).

Urea is a common N fertilizer used in agriculture systems worldwide. It is estimated that more than half of all fertilizer used globally is in the form of urea (Gilbert et al., 2006). The benefit of using urea as a fertilizer is due to its high N content (≈ 46% N), high solubility, and low expense to manufacture, store, and transport (Prasad et al., 1998). However, urea is susceptible to hydrolysis followed by ammonia volatilization (Fenn and Hossner 1985). During hydrolysis, urea N is converted into NH_3, which subsequently reacts with a proton to produce NH_4^+. Under alkaline conditions, the equilibrium of $NH_3 + H_2O \leftarrow \rightarrow NH_4^+ + OH^-$ shifts more to the NH_3 ion, increasing volatilization losses that leads to lower the efficiencies of fertilizer N used by plants. Soil texture and organic C content can also play an indirect role in N gaseous loss. For example, soils with high sand content generally have lower rates of N_2O production than do clay soils (Corre et al., 1996). Leaching intensity is controlled by

soil texture. Lighter sandy soils are more prone to leaching losses than are soils with greater clay content (Hack-ten Broeke and de Groot 1998).

N sustainability

Globally farmers often apply an excess of N as insurance against low yields. This approach can lead to increased losses of N from agriculture systems and poor NUE in plant production systems (Dobermann and Cassman 2004; Goulding et al., 2004). One of the challenges for plant breeders will be to increase NUE in a manner that will reduce production costs and minimize environmental pollution while at the same time meeting both yield and quality measures (Daberkow et al., 2000).

More sustainable agricultural practices that manage N-delivery and its use across a crop production cycle are currently highly sought. For example, the use of split N application procedures, where delivery occurs at a time when plants need N during their life cycle will help to achieve improved NUE that reduces N loss while sustaining or improving yield and quality (Matson et al., 1998). In light of the growing concern about N fertilizer use and its direct economic costs and impacts on the environment, most nations are investigating alternative strategies to make agriculture more sustainable. A reduction in the amount of N fertilizers applied to the field will help to achieve this but at the same time there is a requirement to maintain and or increase yield to meet future food demand. Sustainable agricultural practices, such as N-fertilization based on demand, effective use of crop rotations with N-fixing legumes and the establishment of ground covers and burial of N-rich crop residues are encouraged (Hirel et al., 2007). Others strategies to improve N efficient use are to use genetic modification and/or to breed for new varieties that take up more organic or inorganic N from the soil N and utilize the absorbed or metabolized N more efficiently without compromising yield (Hirel and Lemaire 2006).

Nitrogen and the relationship between yield and grain protein in wheat

Nitrogen is one of the most limiting elements in natural ecosystems (Vitousek et al., 2002) and in most non-fertilised agriculture will limit potential yield. As a consequence, N fertilizers are applied in order to increase yield and improve crop quality. Wheat plants respond favourably to N-fertilization which commonly consists the addition of NH_4^+, NO_3^- or urea alone or in combination with each other. When soil N is low, N fertilizers are often supplied to meet plant demand to maintain yields in most crops. However, when N is applied

in excess, it can often have a detrimental effect on yield due to increased vegetative growth relative to seed production, increased lodging and increased susceptibility to disease and haying-off (van Herwaarden et al., 1998). Although N can be detrimental when used in excess, the proper timing of N fertilizer application and its utilization is important in the context of maximizing growth, yield and quality. Wheat N requirements are greatest during the rapid vegetative growth stages and will most likely benefit from N application/availability during this period.

Grain yield and grain protein content are ultimately determined by the amount of N fertilizer application and its effective uptake from the soil solution. When N becomes available to the plant, primary N uptake seems to be the most promising strategy to enhance the amount of N within the plant to increase both yield and grain protein. This is further supported by the fact growers traditionally benefit from higher returns when grain protein content is high. For wheat, maximum N uptake occurs after tillering and before flowering. N accumulated during these growth stages is used primarily to establish yield potential. N accumulated after flowering has little effect on yield but can increase grain protein content under favourable conditions (Flowers et al., 2007).

Variety selection that takes into accounts both quantity and quality must be adhered to when considering sustainable N management systems. The N harvest index (NHI) is the ratio of seed N to total shoot N and is considered to be a good measure of how efficiently the plant utilizes acquired N for the production of grain protein. However, the total shoot N stored in the grain can vary widely (from 40 to 90%) due to the variation of seasonal precipitation, temperature, wheat species and the type of farming practice. Drought in particular is a significant problem to rain-fed production systems when fertilizer enhanced canopy size becomes a detriment during final seed growth and filling. This response suggests grain protein content has limited genetic heritability that can be actively selected for across a range of growing conditions (Feil et al., 1997). This makes increasing protein concentration a difficult breeding goal (Blackman and Payne 1987). Seed protein cannot adequately form without available N; therefore, a supply of N is a prerequisite for high protein yield. I would assume the best strategy forward is to deal with multiple abiotic stress-related traits together where drought tolerance for example is selected alongside N reallocation to developing seeds.

2.8. N and wheat production

Like most cereal crops, wheat is very sensitive to poor N nutrition but very responsive to N fertilization. Wheat is a fast growing crop where high levels of available N in the soil are often required to meet plant demand. When soil N is low, yield is often reduced due to a reduction in plant vegetative growth (tillering). N deficiency symptoms in wheat are typical of most plants where older leaves yellow (due to less chlorophyll content). Plant demand for N is often met using crop rotations with N_2-fixing legume crops where legume/wheat rotations depend on less N-fertilizer than wheat/wheat or wheat/rice (cereals) rotations.

Increased crop productivity has been associated with a 20-fold increase in the global use of N fertilizer over the past five decades (Glass et al., 2003). N-fertilizer use is expected to increase by at least 3-fold by 2050 (Good et al., 2004). In Australia, total N-fertilizer use was approximately 723,000 tons in 1997 (Jenkinson et al., 2001) and increased in use close to 793,888 tons in 2007 (FAO, 2010). N-fertilizer consumption in Australian agriculture system increased steadily during 1990 and in many cases replaced biological N fixation by pasture legumes as a source of N (Angus et al., 2001). Most wheat production involves N fertilizer application before or at sowing or 6 to 8 weeks after sowing (McDonald et al., 1992). N is available in most soils in the inorganic forms as NO_3^-, NH_4^+ and N_2 and in the organic form as amino acids and urea (Crawford and Glass 1988). The dominant form of N used by higher plants is often NO_3^- which is common in warm, aerated and pH balanced agricultural soils and natural ecosystems (Crawford and Glass 1998). NH_4^+ on the other hand is found in cool soils with low pH and or under anaerobic conditions (i.e. irrigated rice fields (Kronzucker et al., 1999). Thus depending on the soil conditions plant access to N will vary as will selectivity among different plant species (Marschner et al., 2002).

The most common fertilizer used in Australian is urea as it is a cheap form of dry fertilizer and is effective when broadcast, followed by rain after application. N fertilizer application often develops initial root systems that lead to the growth of vigorous root systems that recover N fertilizer and N from the soil than can the root system of an unfertilized crop (McDonald et al., 1989). N uptake depends on root architecture and available soil moisture, so topdressing of N-fertilizer (e.g. urea as used in this project) depends on soil moisture (rainfall) to be present soon after N application. The demand of N-fertilizer by plant at early growth stage is small because plants are young and rely on residual N in the soil. N demand will increase when plants develop into their later stages of development and maturity, this

demand will be met with additional applications. The plants reach peak demand at tillering, heading and grain filling stages. When there is insufficient N at the seedling stage there is a reduction in tillering (increased tiller mortality) and loss of soil water from evaporation, while excessive seedling N causes lodging, foliar diseases and haying-off (van Herwarden et al., 1998). I believe, the use of split application methods that correspond to plant demand at different growth stages is an important strategy that improves N use (NUE) by plant and reduces N loss as a result of volatilization, denitrification or leaching (Kichey et al., 2007; Ehdaie et al., 2010).

Evidence for genotypic or environmental variation in N use in wheat

Many studies have compared crop performance (e.g. yield, quality, NUE) under various fertilizer and management regimes across different environments and growing locations. Unfortunately significant genotype X environment (G X E) interactions exist across the majority of these data sets which are often compounded by differing research methods, management practices, and calculations of NUE (Huggins and Pan 2003; Van Sanford and MucKow 1988). This complicates the development of a general interpretation of how wheat responds to N and the traits, which need to be improved upon to enhance NUE with yield and quality components intact. However, a number of studies have indicated there is genetic diversity amongst wheat lines in their response to low or high levels of N fertilization. For example, Presterl et al., (2003) examined the genotypic variability related to NUE in wheat and showed that some genotypes grew well under low N supply while others didn't. Oritz-Monasterio et al., (1997) found that in all wheat varieties evaluated, both NupE and NutE were reduced at higher N supply, causing an overall reduction in NUE. Le Gouis et al., (2000) found that the genetic variability in grain yield of wheat grown varieties at low N was significant. A recent report by (Quarrie et al., 2007) indicates strong QTLs for yield under low N fertilization conditions exist in hexaploid wheat, and suggests the possibility to improve yield stability by combining QTLs related to yield that are expressed in low N environments. Genetic differences in N uptake and/or grain yield per unit of N applied has also been reported in different crops including wheat, rice, maize, sorghum, and barley (OrtizMonasterio et al., 1997; Muchow et al., 1998; Le Gouis et al., 2000; Presterl et al., 2003; Anbessa et al., 2009; Namai et al., 2009). Under low N supply, a number of studies have indicated modern cultivars had higher yields than old cultivars (Ortiz-Monasterio et al., 1997; Muurinen et al., 2006; Brancourt-Hulmel et al., 2003; Foulkes et al., 1998).

2.9 NUE and Wheat production

The improvement of NUE in wheat is a major challenge necessary to ensure sustainable yields and food security worldwide (Raun et al., 2002). Various alternative production practices have increased NUE relative to more standard high-input cropping practices. For example with crop rotations, the NUE of wheat following a legume is significantly improved than wheat-fallow or wheat-wheat cycles (Badaruddin and Meyer 1994). Similarly, in dry land systems, growing spring barley, corn, and winter wheat in rotation with adequate N-fertilization instead of continuous wheat-fallow improves overall NUE. This suggests that improvements in NUE from crop rotations are due partially to the presence of excess mineral-N in the soil, which minimizes the requirement for N-fertilizer inputs and their associated losses (Halvorson and Reule 1994). It has also been shown that changes to the availability of N to the plant at critical developmental stages can improve NUE. Low rates of N application at tillering followed by higher rates during later stages of shoot development will improve NUE in wheat (Blankenau et al., 2002). Studies have also shown that NUE is higher in tall wheat varieties for dry matter production and in dwarf-wheat varieties for grain production (Singh and Arora 2001). NUE with a high harvest index (dry biomass) and low forage yield were observed in winter wheat varieties (Kanampiu et al., 1997). It was confirmed that adaptation of subsurface placement of N-fertilizer with no-till for winter wheat improved NUE (Rao and Dao 1996). Recently, studies on NUE improvement have been done in modern wheat varieties and have shown an increase in NUE of 14% to 18% in modern UK varieties in response to N supply (Sylvester-Bradley et al., 2009) while other studies showed 24% to 29% increase in NUE in Spanish modern wheat (Acreche et al., 2009). It is important to note though, the differences in NUE reported above were mostly determined by yield responses and not with increased concentrations of N in the plant. Sylvester-Bradley & Kindred (2009) reported that yields of winter wheat in England increased strongly, but NUE only increased slightly from 20 to 24 kg DM. kg^{-1} N over the last three decades. They concluded that NUE improved more through better resource capture than physiological conversion. Moreover, improvements in NUE can also be due to a reduction in cereal diseases that leads to a more vigorous and healthy root system (WWW.hgca.com). Thus, improvements were more dependent on agronomic measures than breeding for enhanced NUE.

2.10. Evidence of NUE productivity

Identifying productivity and quality traits ultimately requires genetic variability to be present amongst parental lines and selections. Evidence of directed genetic improvement of NUE is limited (Kamprath et al., 1982). However, several co-localizations between physiological traits, agronomic traits, and candidate genes were identified in maize, rice and wheat, all related directly or indirectly to the capacity of the plant to take-up or utilize N at a particular stage of its developmental cycle. QTLs from each of these cereals have linked yield and the genes encoding cytosolic GS or leaf GS activity. In maize, Hirel et al., (2001) found that one QTL for thousand kernel weight was coincident with GS1.4 (Gln1-4 locus) and two QTLs for thousand kernel weight and grain yield were coincident with GS1.3 (Gln1-3 locus). Such strong coincidences are consistent with the positive correlation observed between kernel yield and GS activity (Gallais and Hirel 2004). In rice, a co-localization of a QTL for GS activity and a QTL for one-spikelet weight was identified (Obara et al., 2001). In wheat, QTLs for GS activity were co-localized with those for grain N content (Habash et al., 2007). These three studies confirmed previous hypotheses on the key role of the enzyme GS in plant productivity that arose from either whole plant physiological studies, or genetic manipulations (Andrews et al., 2004; Good et al., 2004). In addition, there is evidence of genetic intra-specific variability for NUE in many annual species, including rice (Broadbent et al., 1987), wheat (Le Gouis et al., 2000), and maize (Bertin and Gallais 2001). Previous studies have shown significant differences in NUE improvement among rice genotypes grown in tropical (Tirol et al., 1996), subtropical (Ying et al., 1998), and Mediterranean environments (Koutroubas and Ntanos 2003). NUE improvements have also been observed in dry land systems including spring barley (*Hordeum vulgare* L.) corn (*Zea mays* L.) and winter wheat (*Triticum aestivum* L.) when grown in rotation with adequate N fertilization instead of continuous winter wheat-fallow (Halvorson and Reule 1994). Modern barley genotypes have recently showed improved NUE with increased yields without the need for elevated N application rates (Sylvester-Bradley et al., 2008). In this study it was confirmed that NupE and NutE play a role in improving NUE in the barley. This result is similar to that in maize, where at low N, the genetic variation in NUE for maize related to NutE while at high N, genetic variation in NUE was related to a mix of N uptake and N utilization efficiencies. (Moll et al., 1982).

2.11. Aims/Objectives of the project

This project aim was to:

- Examine the growth response of wheat varieties to low and high N application rates
- Identify genotypic differences in NUE across wheat lines
- Provide information for future research.

CHAPTER 3

Influence of N fertiliser on growth and seed yield of field-grown wheat varieties

3.1. Introduction

Numerous studies have investigated the growth response of wheat lines to different N levels with an aim to show if genetic variation exists in NUE traits including N uptake, N redistribution and N utilisation. A study by (Austin et al., 1977) reported large differences in total N uptake across 43 genotypes of *T. aestivum*. In contrast, (Norman et al., 1992) reported that wheat lines differing in height and canopy architecture (aerial growth) displayed similar total N uptake capacity. While (Guindo et al., 1994) reported that the response to different N application rates was only associated with excessive plant growth at high N rather than with improved plant uptake and/or retranslocation within the plant at lower N supply rates.

This study was conducted to investigate if genotypic differences in NUE exist amongst common wheat cultivars grown in South Australia. Three independent field trials were conducted in 2010 at Mintaro, Pinnaroo and Tuckey, South Australia. The trials involved the use of 6 wheat cultivars (Excalibur, Frame, Gladius, Kukri, Mace and RAC0875). Excalibur, Gladius, Kukri and RAC0875 were chosen as they are four parents of a mapping population that are extensively studied in South Australia for N-related traits, and Frame and Mace have previously been shown to have contrasting N responses in the field (M. Okamoto, personal communication). The selected varieties were cultivated under four levels of increasing exogenous N fertilizer supply (0, 50, 100, and 150 kg Urea / ha). The effect of N fertiliser application was measured based on various plant growth measurements including final grain yield.

3.2. Materials and Methods

Field Site and Environmental Conditions

Field experiments were conducted by Australian Grain Technology (AGT) in South Australia at three locations Mintaro, Pinnaroo and Tuckey in South Australia during the 2010 cropping season. During the trial, South Australia recorded its 3rd wettest year on record, with the state-wide area average precipitation totalling just over one and a half times the long-term annual average. Almost every month had average to above average rainfall across South Australia. For the growing season (May to November) rainfall was above average of 500.8 mm and the mean temperature was 19.5°C; this being equal to the average temperature for the

standard climatological base period of 1961 to 1990 (South Australian Climatic service Centre Bureau of Meteorology). Where rainfall at Pinnaroo (Murray region) was 280 mm, at Mintaro (Clare valley) it was 402 mm, at Tuckey (Eyre peninsula) it was 278 mm from 1st Jun – 30th November 2010 (www.bom.gov.au) (Appendixes 1, 2, and 3). Six spring wheat (*Triticum aestivum*) cultivars were used (Excalibur, Frame, Gladius, Kukri, Mace and RAC0875) across the three sites. Planting occurred on the 27th of May, 4th of June and 15th of June at Tuckey, Pinnaroo and Mintaro, respectively.

Experimental data was collected separately for each of the three sites through the 2010 growing season. Unfortunately heavy rain disrupted continual access to field sites in Pinnaroo and Tuckey, while Mintaro remained mostly accessible. We collected data to compare plant height (cm), grain yield (g/m^2), 1000 grain weight (g), spike number/(m^2), grain %N and %grain protein, total biomass (g), harvest index (HI), and nitrogen use efficiency (NUE). Individual data sets were analysed using two-way analysis of variance (ANOVA). When significance was evident in the data, least significant differences (LSD) was applied at 5% probability level to evaluate differences between N treatment means within cultivars.

Field Trial Design

The field trials were designed by Australian Grain Technology (AGT) with 24 wheat varieties including the six cultivars of our interests as mentioned above (Appendix 8). A split-plot, randomised complete-block-design (RCBD) was applied, consisting of 12 ranges and 24 rows with four N fertilizer rates. Wheat genotypes were in sub-plots to minimize border effects from the different N fertilizer rates with three replicates for each cultivar and N treatment (Figure 3.1). Thus, we focused on 72 plots each site (6 wheat cultivars and 4 N treatments with 3 replicates) (see Figure 3.1 for field trial design). The net size of a split-plot was 1.2 m wide x 3.2 m long consisting of 5 X 20 cm seeding lines. Seeding rate was 150 seeds /m^2 and planting depth was 2 cm.

	Ranges												
Rows	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**Nitrogen treatments**
1								M	K	F			**N1**
2							E				G	R	**N1**
3	K	G									F	R	**N4**
4		M	E										**N4**
5	K	E											**N3**
6	F					R	M				G		**N3**
7													**N2**
8		E	M		K	R	F			G			**N2**
9							G	M	E		R		**N3**
10				K								F	**N3**
11				F					M		K		**N2**
12			G			R	E						**N2**
13		E		K								M	**N4**
14		G		F		R							**N4**
15			G				E						**N1**
16		M	R		K				F				**N1**
17											F		**N4**
18		E	R		K					M	G		**N4**
19		F	R	M				K					**N3**
20										G	E		**N3**
21						E							**N1**
22	K						G	M		R		F	**N1**
23		G				E	F	K				R	**N2**
24		M											**N2**

Figure 3.1: Field trial design.Trial includes 12 ranges and 24 rows, selected six wheat varieties from 24 varieties, using three replicates for each of the cultivars and for the four rates of N-treatments, cultivars are: (E) Excalibur, (F) Frame, (G) Gladius, (K) kukri, (M) Mace, (R) RAC0875. The N-treatments are: (N1=0, N2=50, N3=100, N4=150 kg urea / ha).

Nutrient Fertiliser Remediation

An initial soil test was conducted by AGT across each site to calculate the level of available nitrogen (NH_4^+-N and NO_3^--N) and other chemical and physical characteristics of each of the three sites in(Appendix: 5). With this information, a basal fertilizer 90 kg/ha of (di-ammonium phosphate (DAP) consisting of nitrogen 16% N) was applied at Mintaro, Pinnaroo and Tuckey. For each site the plants were provided four levels of N-fertiliser above the basal fertiliser amount (0, 50, 100 and 150 kg urea/ha) at sowing. Urea (46% N) was used as a source of N at the three sites.

Plant Growth, Harvest and Processing of Tissues for total N analysis

Plant growth measurements were taken at harvest and tissues collected. Plant analysis included:

1. Plant height (cm) at maturity, ten plants was selected randomly in each split-plot for height measurement and means were taken for statistical analysis.

2. Total biomass at harvest were taken for each split-plot at 0.6 m^2, bagged and weighed after being air-dried.

3. Yield components recorded for each split-plot included, grain yields per 0.6 m^2 - converted to g/m^2, 1000-grain weight (g), head numbers.

4. Grain nitrogen content was measured by gas chromatograph connected to an isotope ratio mass-spectrometer.

5. Estimates of grain protein content based on %N measurements.

The crop was harvested individually per 0.6 m^2 plot by hand in December 2010 and the samples were bagged and air-dried. Grain and dry matter were prepared by threshing spikes and shredding aerial foliage. Sub-samples (5 g) of each were dried in an oven (40°C) for 48 hours. Tissues were ground to a fine powder using a grinder machine (Labtech Essa – LABTECH ESSA LM1- PTY. LTD), grain N% was determined from a 3-4 mg subsample of each replicate. Tissues were transferred into tin capsules and processed through an Isotope Ratio Mass-spectrometer (SerCon Hydra 20-20) operated by the analytical service laboratory at the Plant Research Centre, Waite Campus, University of Adelaide. Grain protein content was estimated by multiplying total grain %N content with the wheat global standard factor of 5.71 (Lopez-Bellido et al., 2004).

Statistical analysis

The data was analysed using a split-plot two-way general ANOVA (Genstat) to test for significant differences between cultivars and nitrogen treatments across the three sites. Nitrogen treatment, cultivar and nitrogen x cultivar effects were tested for significance ($p<0.05$). Multiple comparisons (least significant difference, protected) were then tested post-hoc across main effects or when significant interactions existed between nitrogen and cultivar treatments.

3.3. Results

Plant height (cm)

At both Mintaro and Tuckey (Pinnaroo was not assessed) there was no significant interaction between cultivar and N treatment on final plant height (P=0.977 and 0.687, respectively - see Appendix 4). At each site, plant height did vary between cultivars but plant height only varied with N-treatments at Tuckey (Fig 3.2 A, B).

Plant head number (spikes) (m^2)

At the Mintaro and Pinnaroo sites (Tuckey was not measured) there was no interaction between N treatments and cultivars (P=0.199 and 0.059, respectively – Figure 3.3 A, B and see Appendix 4). At Mintaro, there was significant variability for the cultivar main effect but not at Pinnaroo (P=0.030 and 0.059).

Grain Yield (GY) (g /m^2)

At all three sites there was no significant interaction between cultivars and N treatments to grain yield (P=0.623, 0.276 and 0.733, respectively – see Appendix 4). At Mintaro and Tuckey, grain yield responded to N treatment as a main effect while all three sites displayed a cultivar main effect (Figure 3.4).

Thousand Grain Weight (TGW) (g)

There were no significant interactions between cultivar and N treatments to TGW at each of the three sites (P=0.392, 0.460, 0.071, respectively – see Appendix 4). At all three sites both Nitrogen and Cultivar showed variability in TGW (P<0.05) (Fig 3.5).

Total Grain Nitrogen (TGN) (%) and calculated % protein

Grain quality (N and protein) is an important component of final grain yield and its final commercial value. Across all three field sites there was no significant interaction between cultivar and N treatment (see Appendix 4) to both TGN and the calculated %protein. Overall main effects to N were found at Mintaro but not across Cultivars at the three sites Figures 3. 6, Figure 3. 7).

Total biomass (g / m^2)

Total biomass is the total weight of the above ground parts of the plant including, grains, stems (tillers), and leaves harvested per unit area. Across all cultivars at both Mintaro and Pinnaroo (Fig 3.8 A, B) shoot biomass per harvested area did not vary with N treatments (P>

0.05, Appendix 4). Unfortunately, total biomass data was not collected at Tuckey because of heavy rains.

Harvest Index (HI)

Harvest Index is the ratio of grain yield to above ground total biomass produced in a defined unit of area. At both Mintaro and Pinnaroo (data not collected from Tuckey) there was no interaction between nitrogen treatment and wheat cultivars ($P>0.05$) (Fig 3.9 A, B), (Appendix 4)

Nitrogen Use Efficiency (NUE)

In this study, we adopted the NUE definition proposed by (Moll et al., 1982). Nitrogen Use Efficiency (NUE) is referred to as grain yield produced per unit area of available N in the soil (residual N + applied N) in the same unit of area.

NUE = GY (kg / ha) ÷ (residual N + applied N) (kg / ha)

Residual N in the field soil was calculated on the base of the equation below:

Available soil N in kg/ha = nitrate (mg/kg) × bulk density of soil (g/cm3) × test depth/10 (www.bcg.org.au) (Appendix 6).

Soil bulk density values are known for various soil types (www.bettersoils.com.au), and are shown in (Appendix 7).

Across the three sites, the variation across both cultivars and N treatments was found to be significant ($P<0.05$, Appendix 4). Like all other assays measured, there was no significant interaction between N and Cultivar ($P>0.05$). With most cultivars and across each of the sites, NUE decreased with increased N application (Figure 3.10 A, B, C). This is a common result associated with the equation we used to calculate NUE. A comparison was made across sites where NUE at N1 vs. N2 (N4/N1), N1 vs. N3, N1 vs. N4) was calculated for each cultivar and averaged across the three trial sites (Figure 3.11). In theory those cultivars where NUE remains elevated at higher external N may suggest an improved capacity to grow and utilise N, potentially a higher NUE capacity. In the majority of cultivars we found no significant change in NUE maintenance as external N increased, with the exception of Mace (NUE2 vs. NUE4, $P<0.05$) which had a significant drop in NUE capacity at elevated N (Fig 3.12).

3.4 Summary

There were difficulties in identifying lines which consistently performed well across the three field trials. In general, we found little differences between the field sites in Grain %N in response to increasing N provision (Figure 3.6). Grain yield at both Pinnaroo and Tuckey was vastly different from that of Mintaro achieving only ~50% of the Mintaro grain yield and shoot biomass at Pinnaroo was approximately half that of the Mintaro site. The result further highlighted the strong impact of the poor growing conditions at Pinnaroo and Tuckey which overrides the potential impact of increased N provision. Although, at the Tuckey site, the results showed that all cultivars responded more in grain yield to N treatments compared to the other two sites (Figure 3.4C). Also noticeable N response in grain N observed in Pinnaroo (Figure 3. 6B).

In general, NUE was at its highest level at N1 and decreased sharply as N fertiliser application rates increased up to and including N4 (Figure 3.10). The data also suggests there was little change in NUE capacity between varieties across the three field sites.

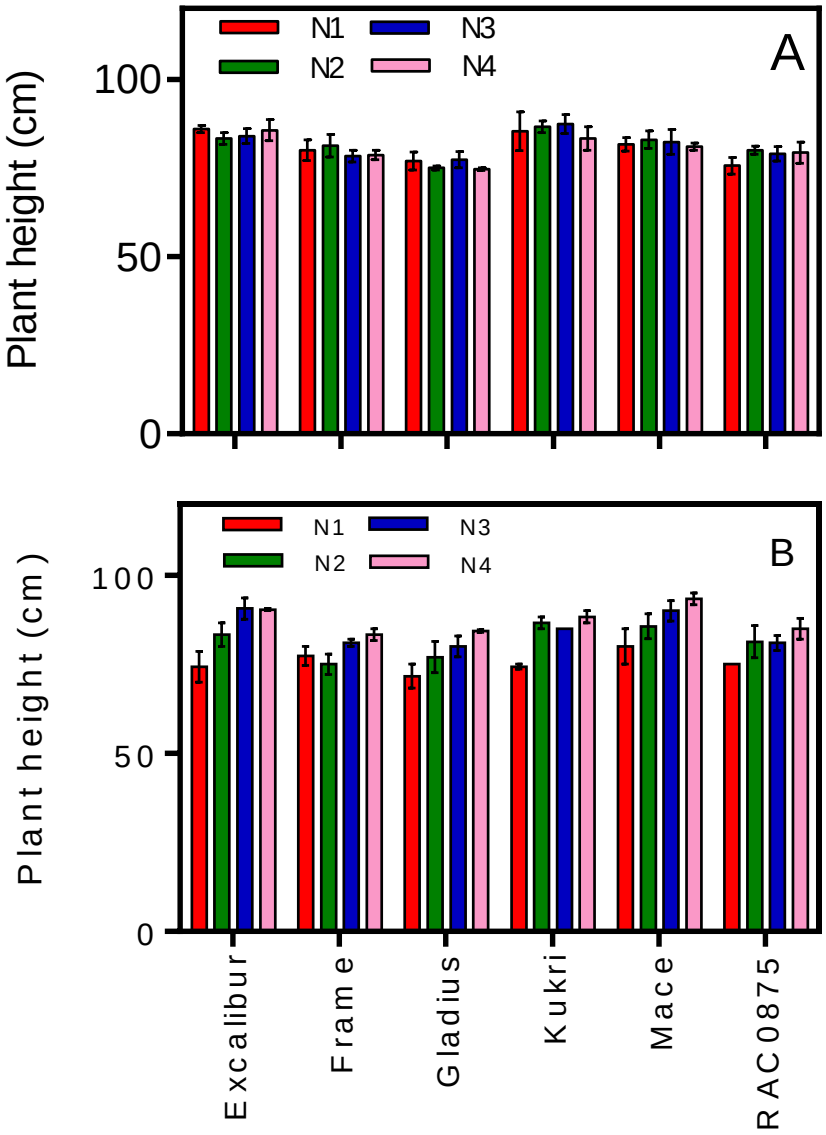

Figure 3.2. Plant height (cm) of wheat varieties affected by N treatments. (A) - Mintaro, (B) - Tuckey. Data represents mean ± SE (n=3).

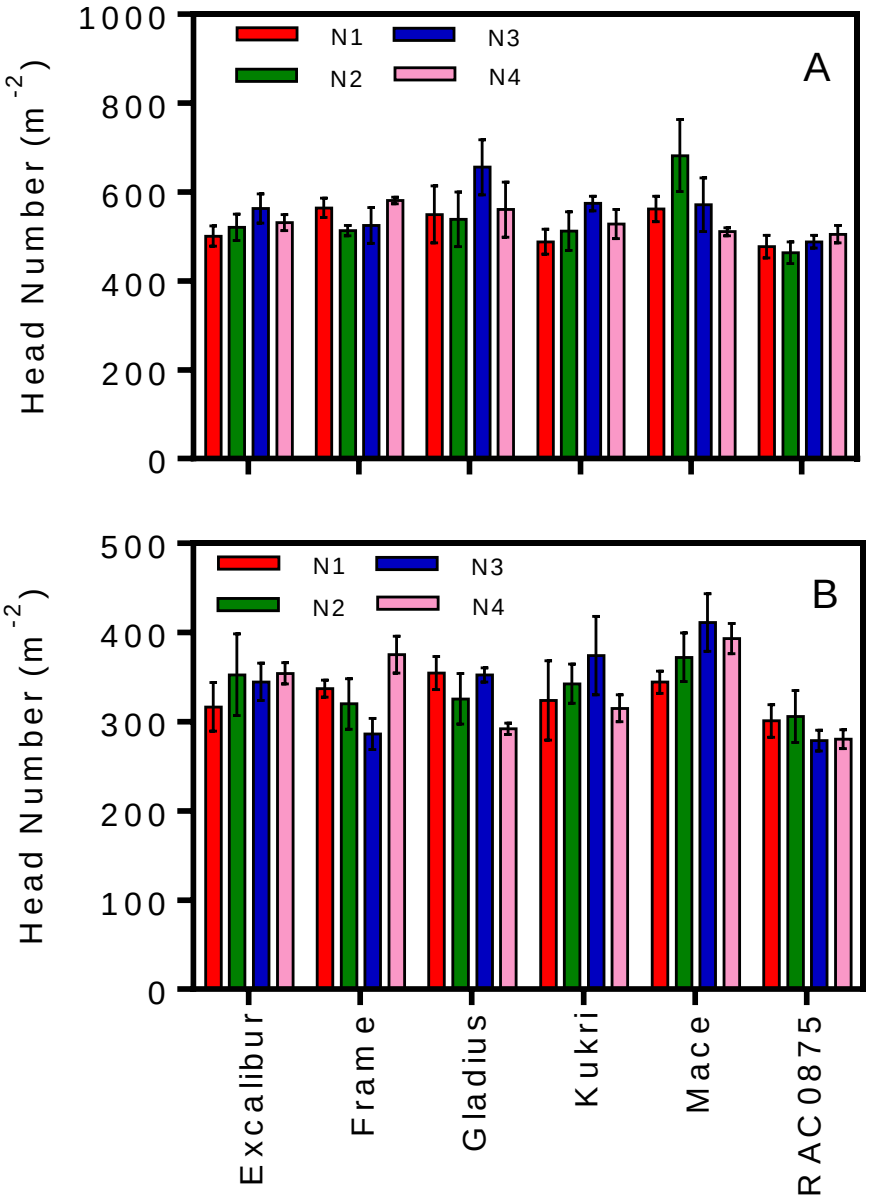

Figure 3.3. Head number (spikes) of wheat varieties affected by N treatments. (A) - Mintaro, (B) - Pinnaroo. Data represents mean ± SE (n=3).

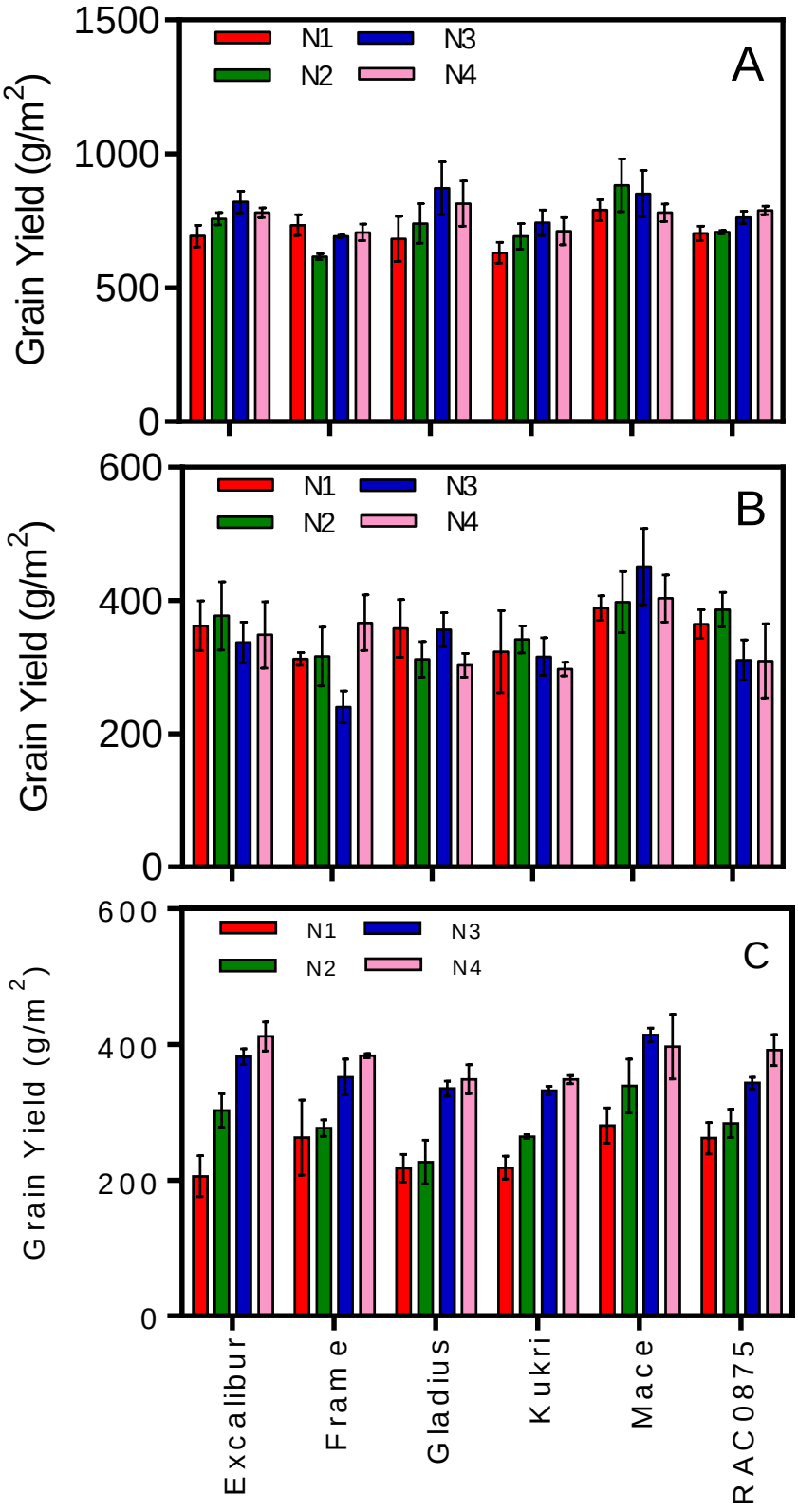

Figure 3.4: Grain yield affected by different N treatments. (A) - Mintaro, (B) - Pinnaroo, (C) - Tuckey. Data represents mean ± SE (n=3).

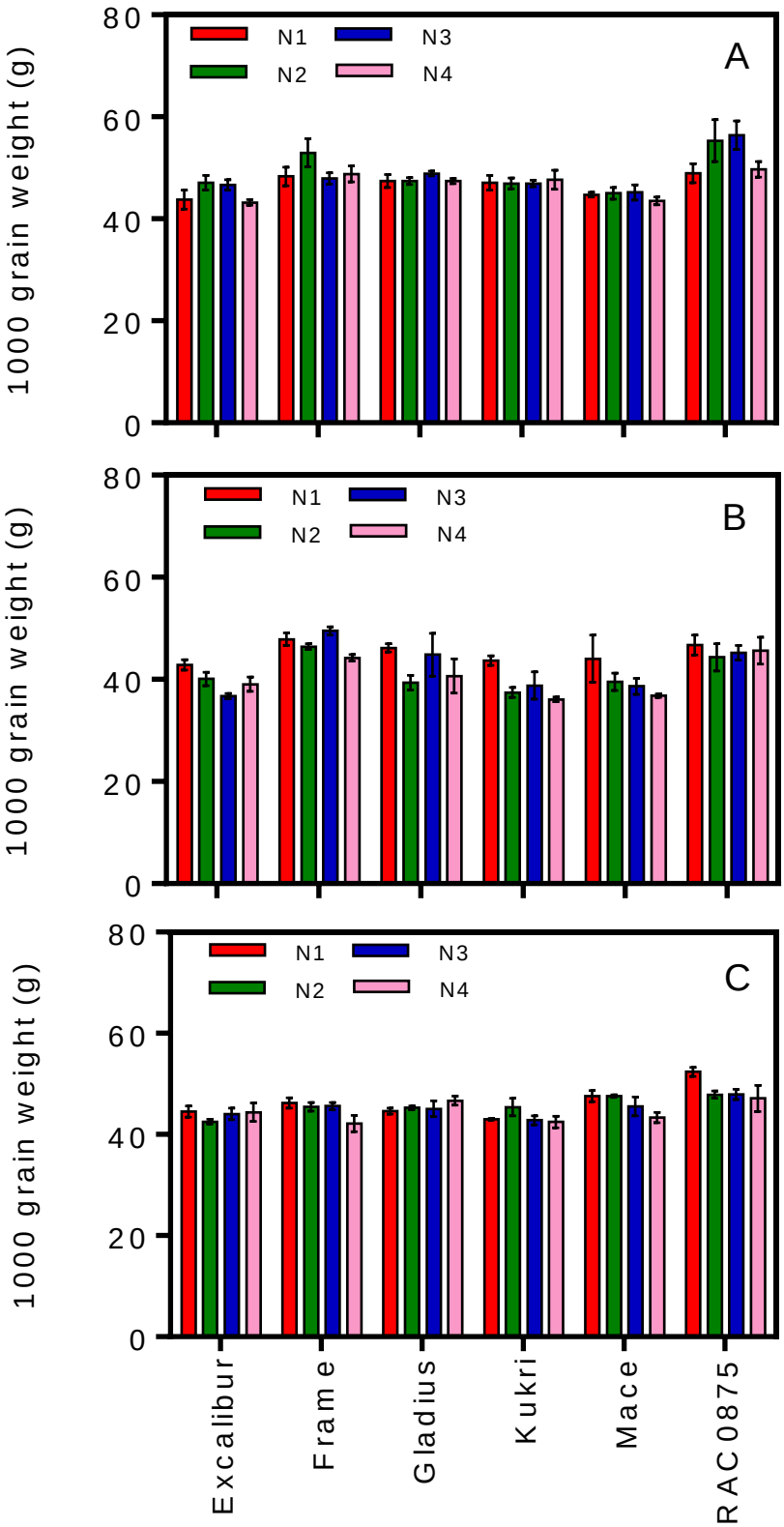

Figure 3.5. Effect of N treatments on thousand grain weight (TGW) of wheat cultivars. (A) - Mintaro, (B) - Pinnaroo, (C) - Tuckey. Data represents mean ± SE (n=3).

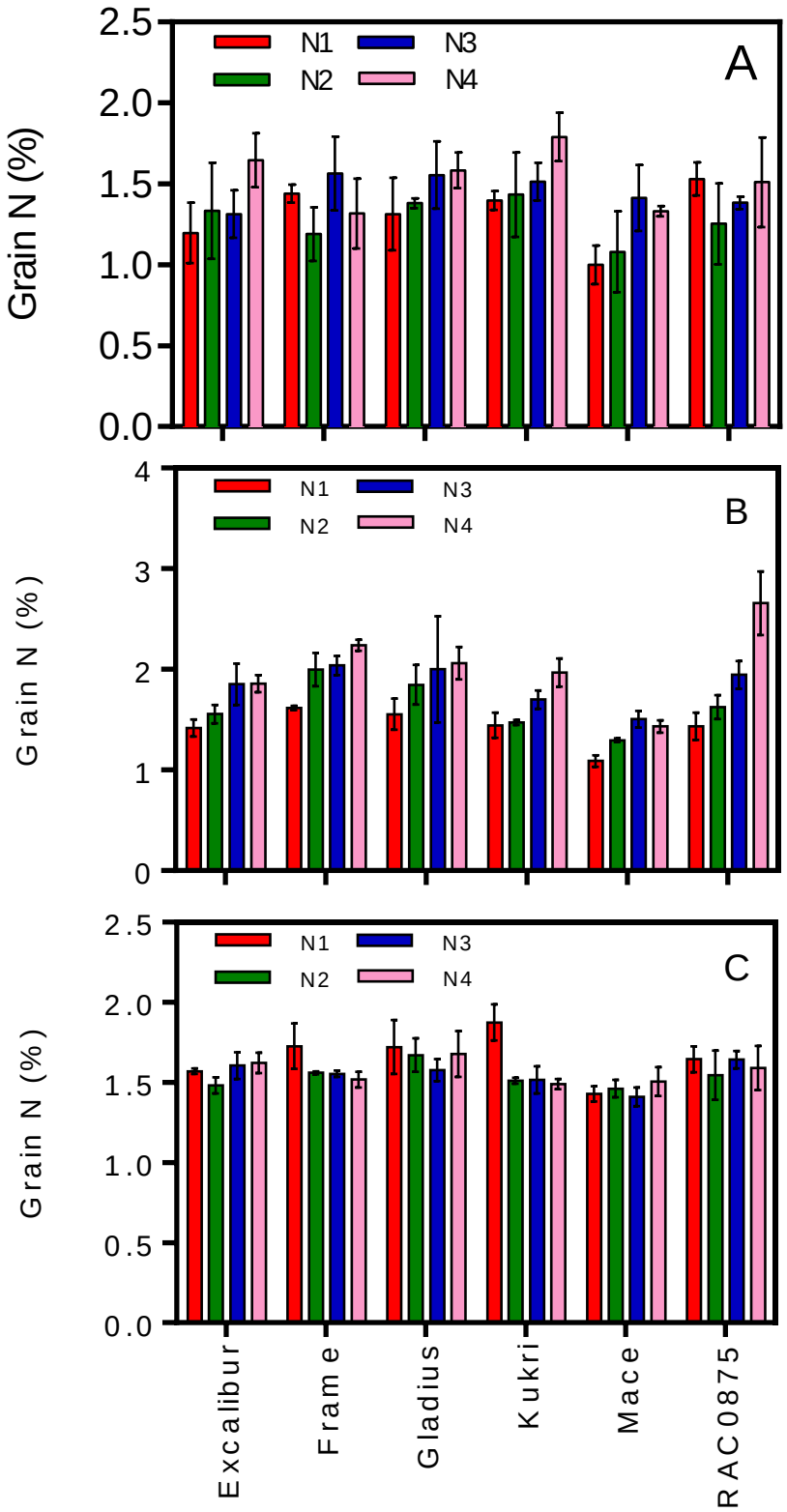

Figure 3.6. Effect of N treatments on Grain (N %) content. (A) - Mintaro, (B) - Pinnaroo, (C) - Tuckey. Data represents mean ± SE (n=3).

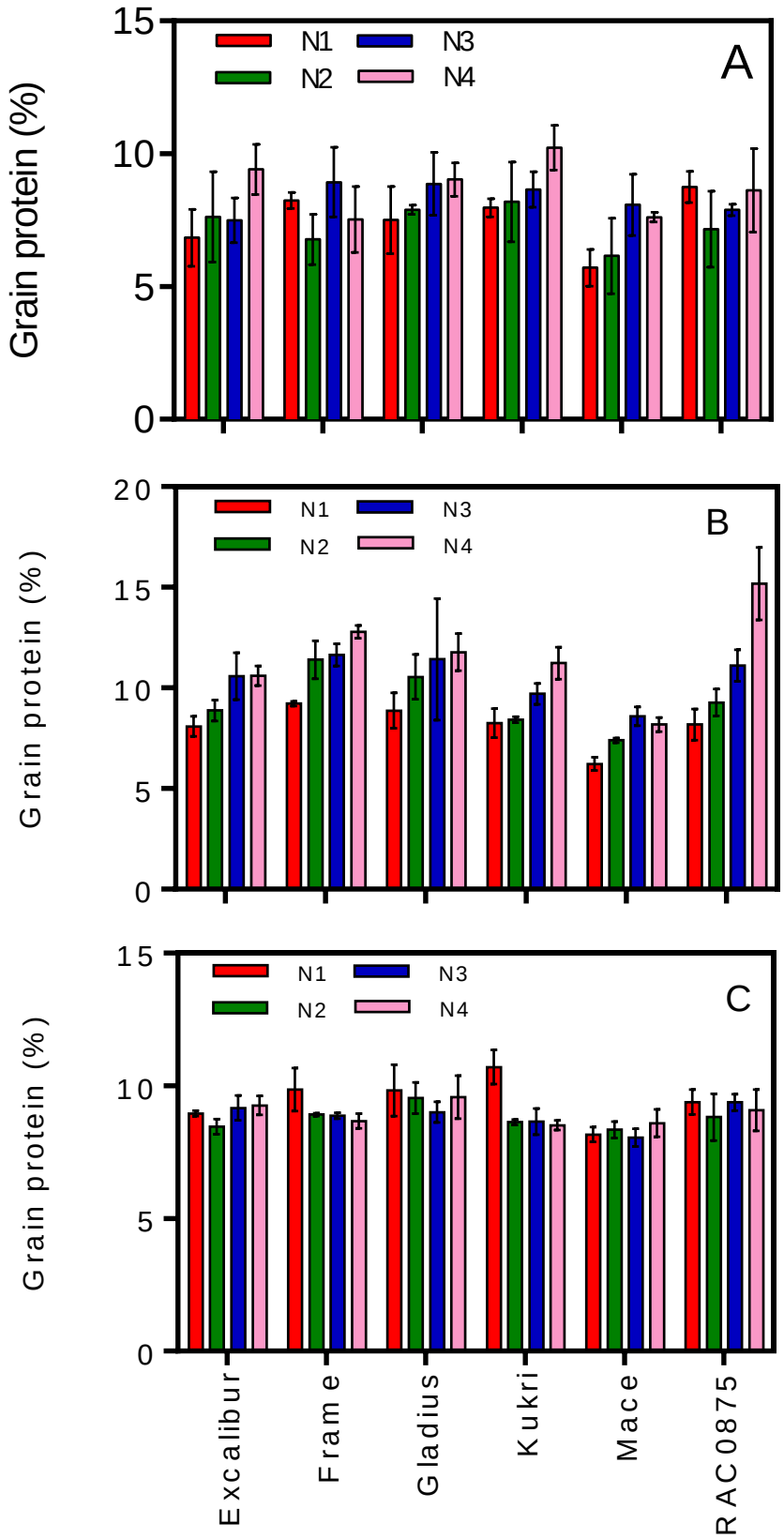

Figure 3.7. Effect of N treatments on Grain Protein (%) content. (A) - Mintaro, (B) - Pinnaroo, (C) - Tuckey. Data represents mean ± SE (n=3).

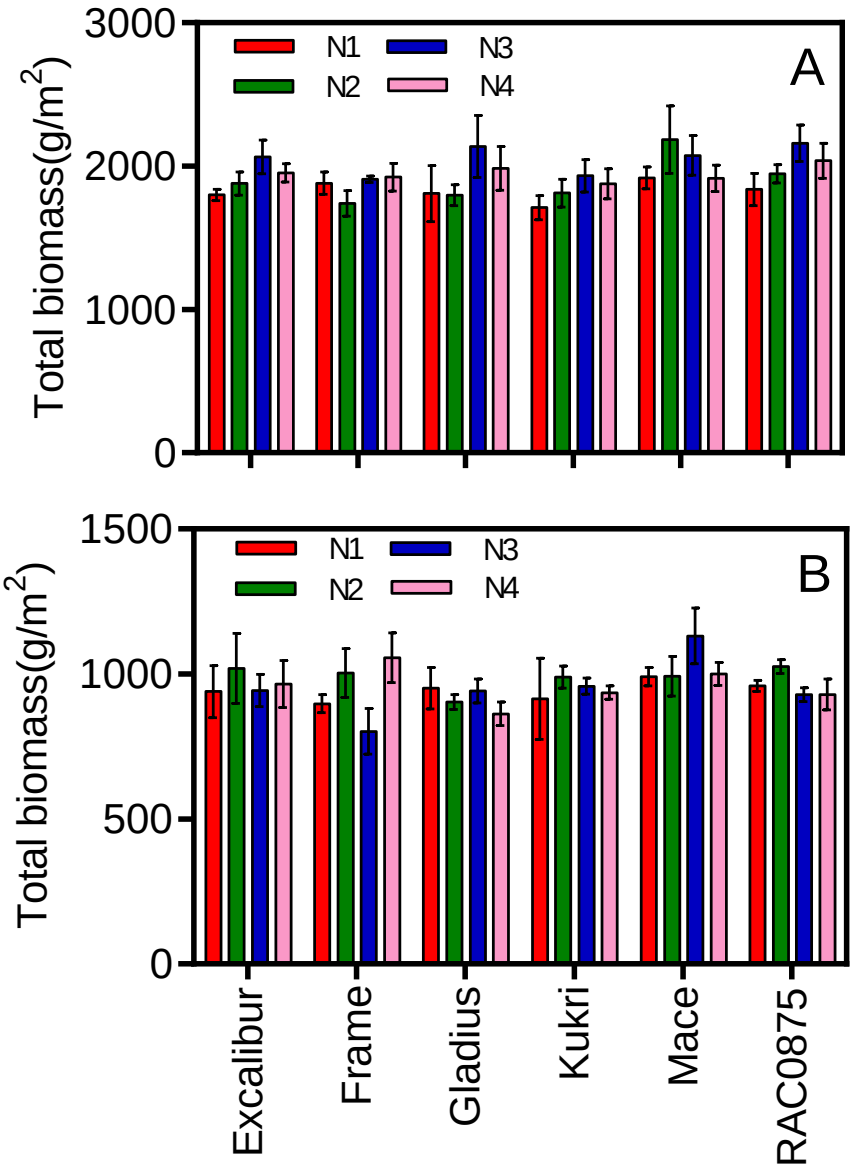

Figure 3.8. Effect of N treatments on total biomass. (A) - Mintaro, (B) - Pinnaroo. Data represents mean ± SE (n=3).

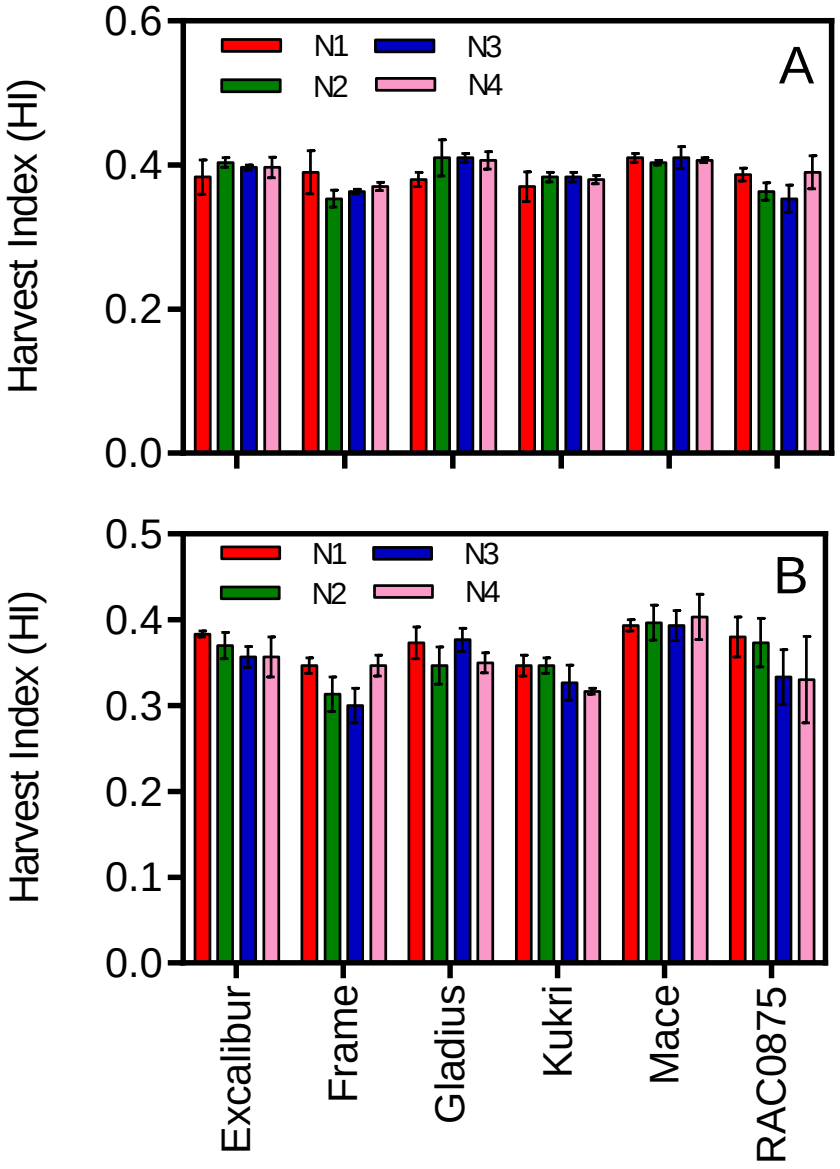

Figure 3.9. Effect of N treatments on Harvest index (HI). (A) - Mintaro, (B) - Pinnaroo. Data represents mean ± SE (n=3).

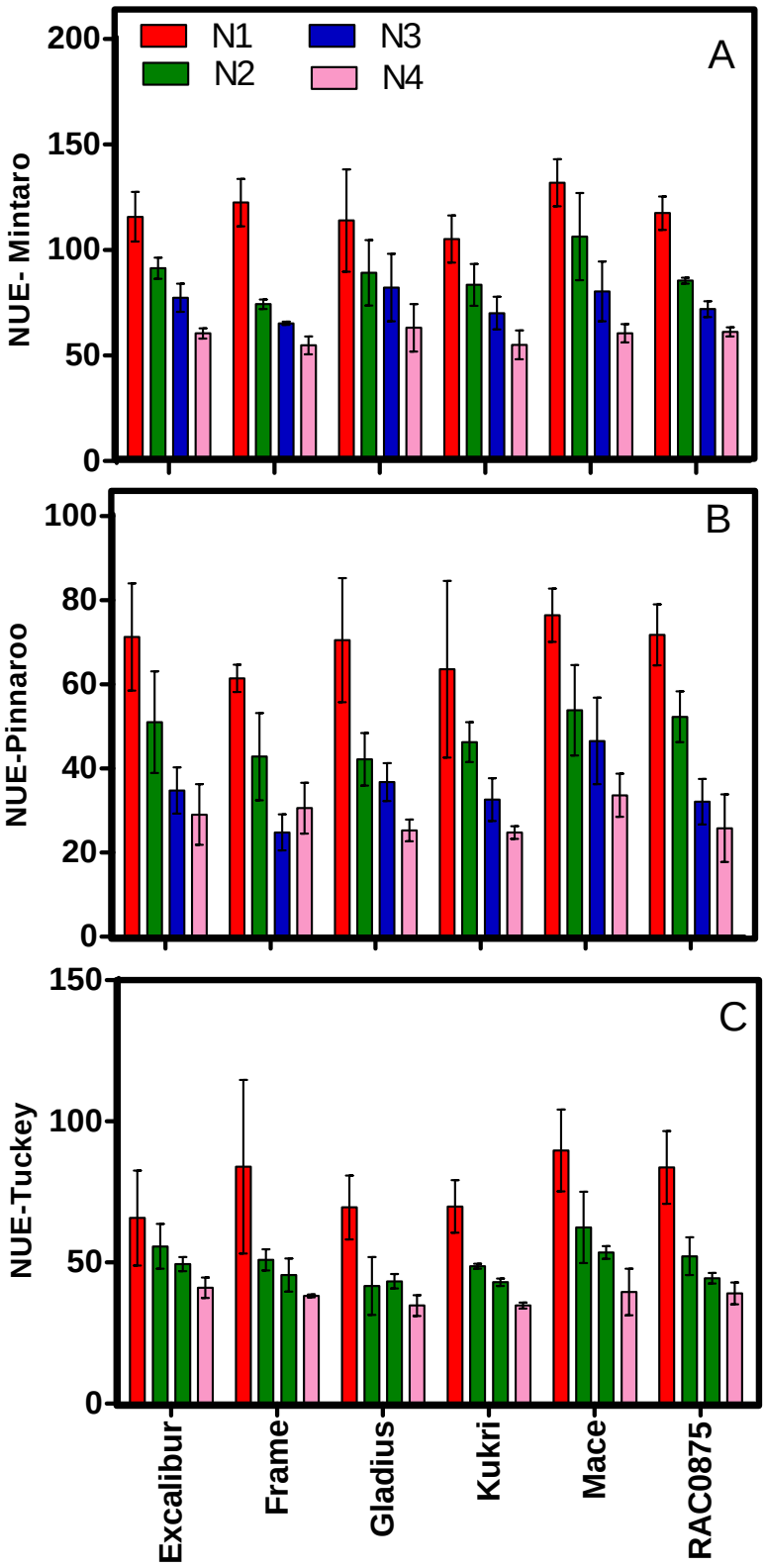

Figure 3.10. Effect of N treatments on (NUE) in wheat varieties at the three sites: (A) - Mintaro, (B) - Pinnaroo, (C) - Tuckey. Data represents mean ± SE (n=3). NUE = grain yield (kg/ha) / available soil N (kg/ha).

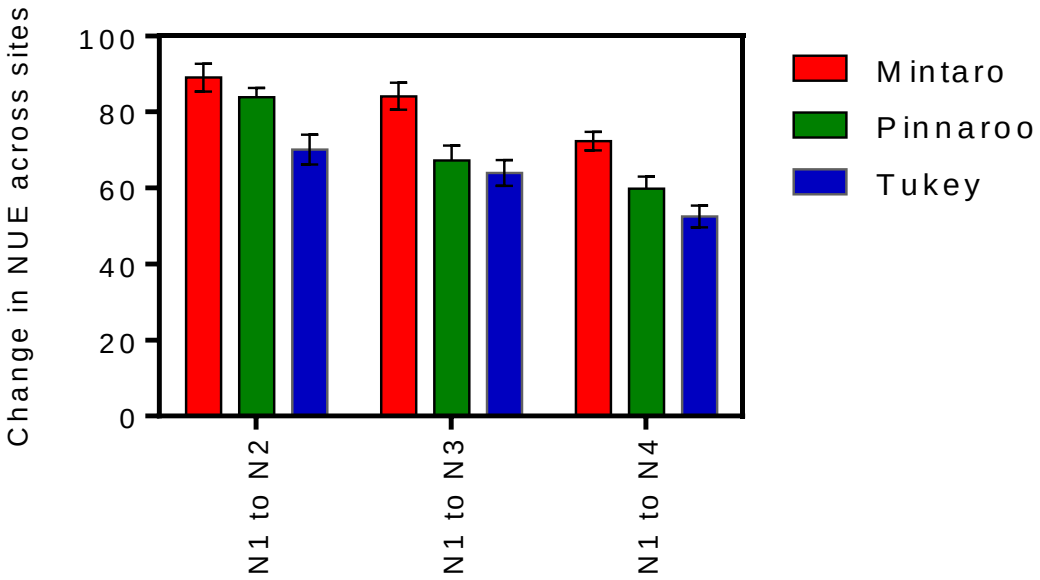

Figure 3.11. . A comparison for NUE in tested varieties with elevated N across the three sites.

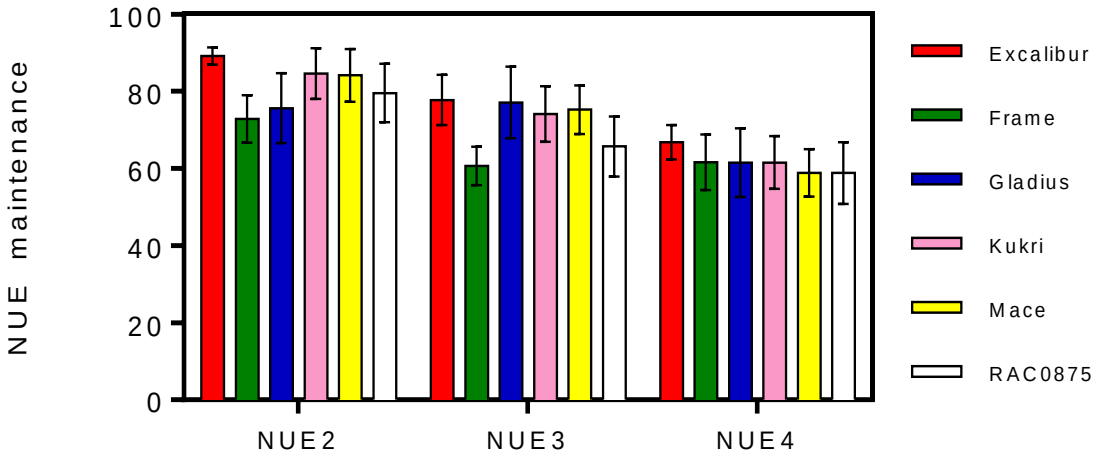

Figure 3.12. Change in NUE (maintenance) as external N increased.

CHAPTER 4

Analysis of N-dependent growth responses of wheat cultivars cultivated under controlled glasshouse conditions

4.1 Introduction

This study was conducted to investigate the growth response of six wheat varieties (the same varieties used in the field experiments as described in Chapter 3) to different nitrogen rates supplied under controlled conditions within a glasshouse. Two separate experiments were carried out using three large soil-based bins with nitrogen fertilizer treatment (low, medium and high N levels of applied urea fertiliser). Unfortunately pot replications were not possible due to space constraints and therefore statistically relevant inferences from the data is limited. However, this study has led to a technical development tool for the cultivation of wheat lines in large soil bins under controlled glasshouse conditions.

4.2 Materials and Methods

Plant Growth and Culture

The experiments were conducted in a glasshouse at Waite campus, Adelaide University between July to November 2010 (Experiment 1), and from February to June 2011 (Experiment 2). Plants were cultivated in large bins (110 cm x 92 cm x 60 cm, 600 L) containing a soil mixture (approximately 70% sandy loam, 30% organic material (coco-peat), soil residual N (see Appendix 8). Plants were grown in a temperature controlled glasshouse where day temperatures ranged between 25-30°C and night temperatures 15-20°C. Plants were provided supplemented light using metal halide 1000 W bulbs. For both experiments lights were used between 6:00-9:00 am and between 4:00 – 7:00 pm.

Experimental Design

Six wheat cultivars (Excalibur, Frame, Gladius, Kukri, Mace and RAC0875) were used (Appendix 9). Seeds were directly sown into the soil of each bin following a grid layout, which included rows of 40 cm long with 14 cm between rows and 5 cm between seeds in two pseudo replicates. The seeding depth was 3 cm. Two wheat cultivars were also used as border lines (shown below in Figure: 4.1). Measurements were taken on a per row basis.

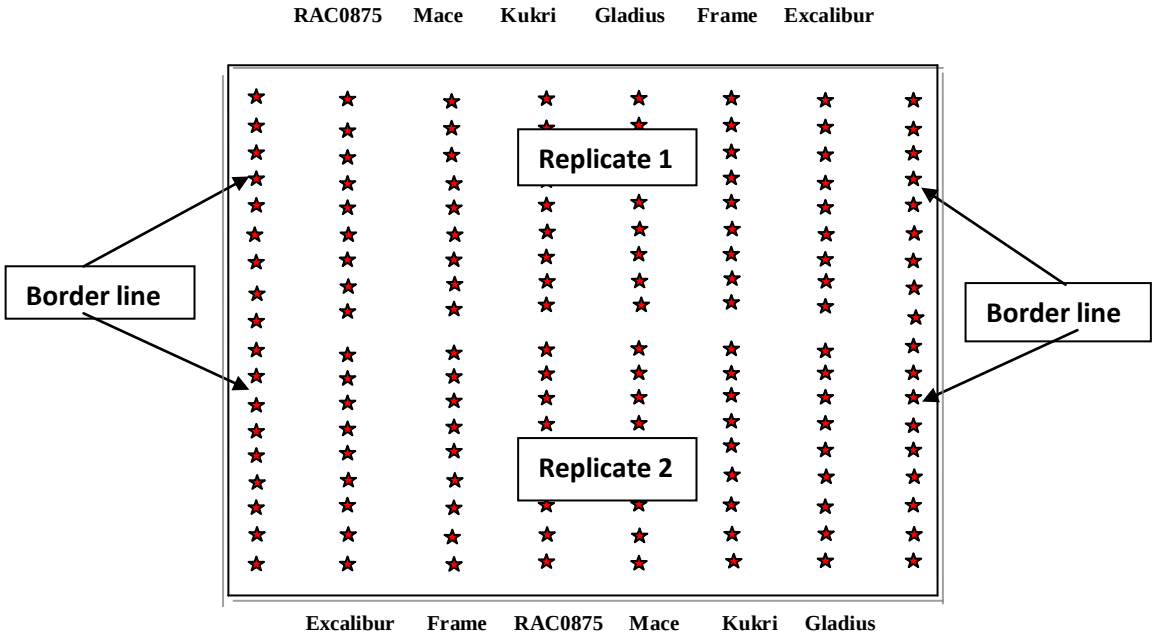

Figure 4.1. Glasshouse Experiment, pot size 600 L (110 cm x 92 cm x 60 cm) including row of 40cm length and 14 cm between rows, and 5 cm between seeds.

Nitrogen Treatments

An initial soil test was done for the soil mixture (Soil Analytical Laboratory – CSIRO, Wait Campus) to estimate the level of available nitrogen and the soil physical characteristics (see Appendix 7). Three N treatments were implemented across three bins. At planting, soil of each bin was provided with starter fertilizer of 2 g N/m^2, which was the base low N treatment. For the medium and high N treatments, a second N-fertilizer treatment was applied. Urea (46% N) was supplied at a rate of 11.96 g $urea/m^2$ (equivalent to a final N addition of 75 kg N ha^{-1}) for the medium N treatment, and 28.26 g $urea/m^2$ (equivalent to a final N addition of 150 kg N ha^{-1}) for high N treatment. Micronutrients were also provided seven weeks after planting to avoid nutrition deficiencies (Appendix 10). Each bin received a supplementary fertilisation of Fe (EDTA) 60 ml per bin when iron deficiency was diagnosed. Plants were watered by hand three times per week until maturity.

Analysis

Non-destructive plant measurements recorded during growth included plant height, head number and chlorophyll content. Leaf chlorophyll was measured using a SPAD meter (Konica Minolta SPAD – 502 Plus, Osaka, Japan) at Zadoks stage 31 across multiple regions

of the leaves. The SPAD meter measures the transmittance of infrared light at 920 nm and the transmission of red light at 650 nm. Leaf chlorophyll absorbs light strongly at 650 nm, while no absorption occurs at 920 nm. Prior to final harvest, plants were cut off from watering four weeks and allowed to dry-off in the glasshouse environment. After harvest, the plants were separated into heads and the remaining aerial biomass. Grain and its component measurements included the number of spikes/row, grain yield (g/row), and 1000-grain weight (g) per cultivar treatment. For total %N, a representative sample of grain per row was oven dried at 40°C for 48 hours and ground to a fine powder. Sub-samples of 3-4 mg were placed into tin capsules and %N determined by Mass-spectrometry in the analytical service laboratory at the plant research centre, Waite Campus, Adelaide University. Grain protein content was estimated by multiplying total grain %N content by the wheat global standard factor of 5.71 (Lopez- Bellido et al., 2004). Harvest index (HI) was calculated as the ratio of grain yield g/row to above ground biomass produced per g/row. NUE was calculated as described in Chapter 3. As the experiment lacked technical replications and instead contained pseudo 'in pot' row replicates, the data was not analysed for statistical significance between treatments and cultivars.

4. 3. Results

Plant growth

In both experiments 1 and 2, we observed little difference in plant height between cultivars and nitrogen treatments (Fig 4.2 A, B).

Plant head number (spikes) per row

In experiments 1 (Fig 4.3A), plant head number was consistent across the nitrogen and cultivar combinations while head number was more variable in the second experiment. Although we can't test this statistically it would appear cultivars such as Excalibur and Frame responded to the nitrogen treatment with increased plant head numbers (Fig 4.3B).

Plant leaf chlorophyll content

Plant leaf chlorophyll content and leaf nitrogen (N) content was measured at GS31 using a SPAD 502 meter. In experiment #1 and #2 chlorophyll contents were comparable across the N treatments and cultivars (Fig. 4.4A,B).

Grain Yield (GY)

In experiments #1 and #2 grain yields were variable between cultivars and nitrogen treatments. There was no clear trend in response to the N treatments although it would appear Excalibur may have responded positively to the extra N supplied (Figure 4. 5).

1000 Grain weight (TGY)

In experiment #1 and #2 there was little change in TGW between cultivars and N treatments. In experiment #1, Excalibur displayed a strong response to N treatment (Fig 4.6a).

Total Grain Nitrogen (TGN) and Grain protein content

In experiment #1 there was clear trend with increased Grain N and Grain Protein from the N1 to N3 treatments (Fig 4.7A). In contrast, in experiment #2, there was no clear response to N treatment. It is worth noting that for each N1 treatment there were higher levels (0.5-1%) TGN and protein in experiment #2 than in experiment #1.

Total biomass and Harvest Index (HI)

Total biomass was measured as the total weight of above ground tissues per row. Shoot biomass includes grain, tillers (stems) and leaves. In experiment #1 and experiment #2 there was no clear trend between cultivars and N treatments to total plant biomass or to that of harvest index. There would appear to be variability between cultivars but minor changes between the three N treatments.

Nitrogen Use Efficiency (NUE)

NUE was calculated as done for the field trials (Chapter 3) (adopted by Moll et al, 1982). The initial soil residual N was uniform across the three bins as the soil was mixed together before distributing into the three bins. Available soil N was calculated:

Available soil N kg/ha = nitrate (mg/kg) X bulk density of soil (g/cm3) X test depth/10 (www.bcg.org.au) (Appendix 5).

In both experiments, all cultivars had the highest NUE at the low N level (N1) which decreased as N input increased. This was similar to that observed in the field trials (Chapter 3) (Fig 4. 11A, B).

4.4 Summary

There was a range of phenotypical differences to N treatment in the glasshouse experiments. In general most measurements failed to show significant relationships between N treatment and cultivar. There was a strong response in grain %N to increasing N provision when plants were grown in the spring/summer season (i.e. Experiment 1) but not when the plants were grown later in the season (Experiment 2). However, grain yield and shoot biomass were found to be roughly similar across the N treatments and the two growing periods. In general, NUE measured in experiment #1 and #2 reduced as external N addition increased.

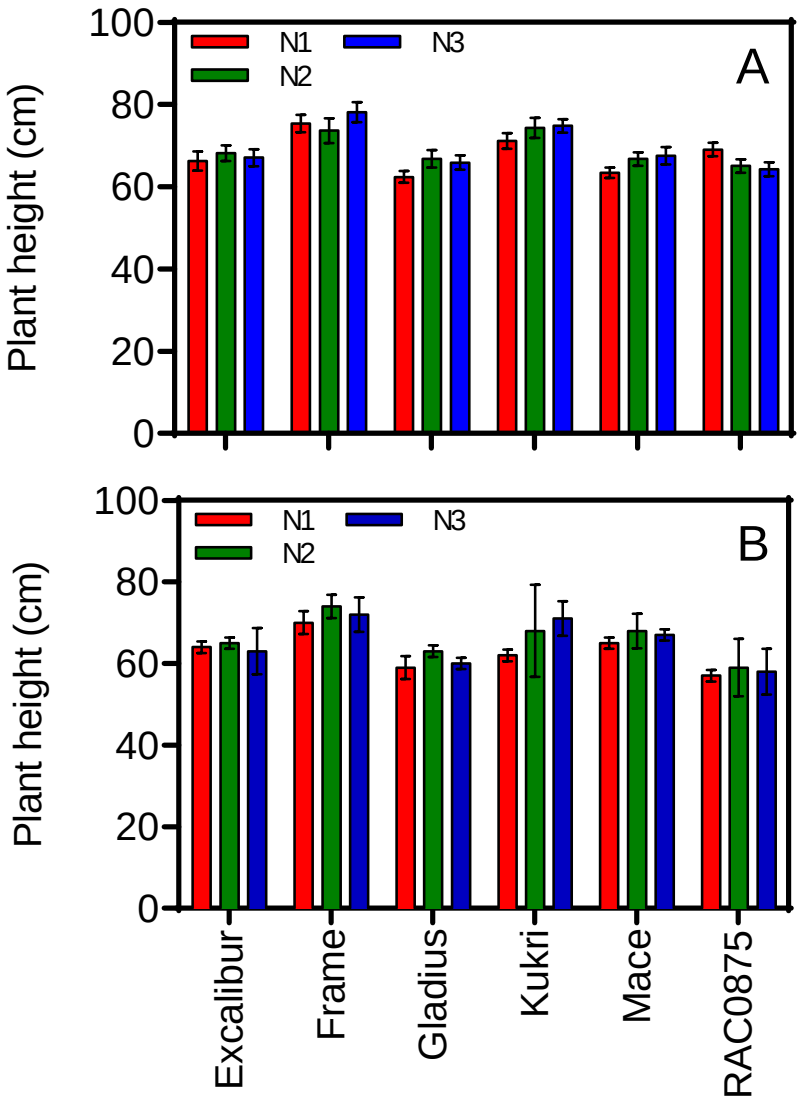

Figure 4.2. Plant height (cm) in wheat varieties affected by N treatments. (A) - Experiment #1, (B) - Experiment #2. Data represents mean ± SE (n=2).

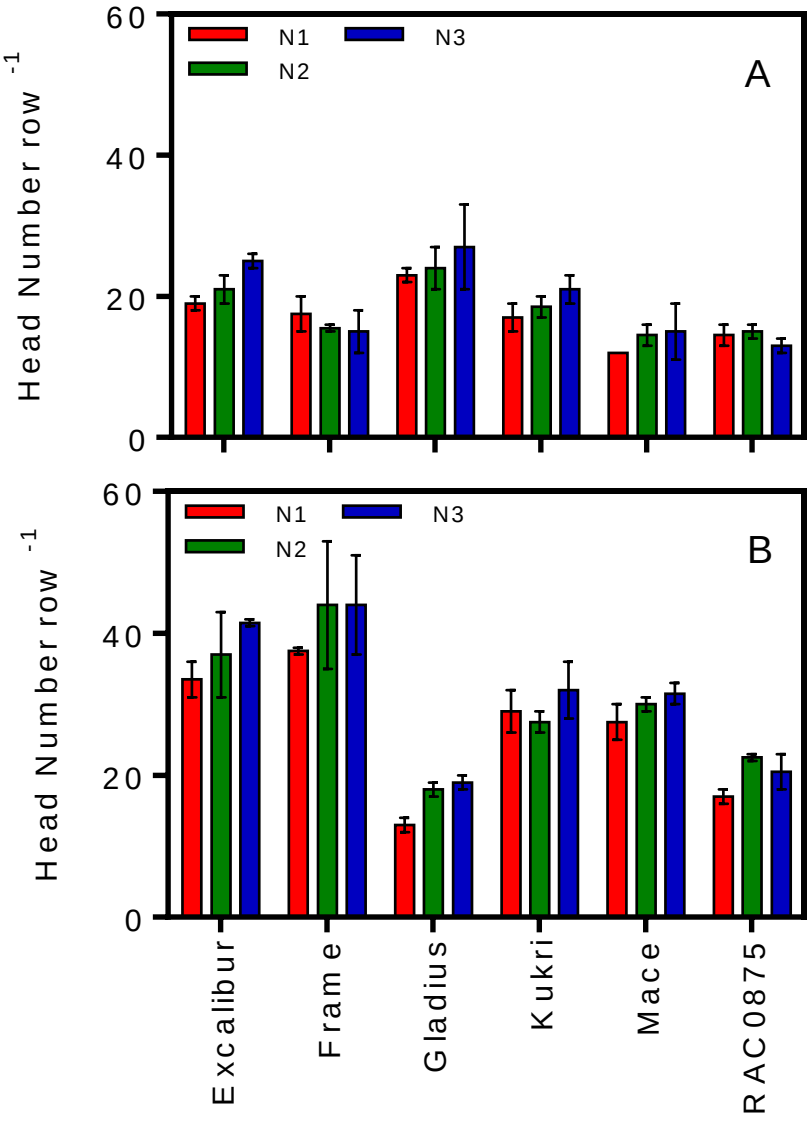

Figure 4.3. Plant head number (spike) of wheat varieties affected by N treatments. (A) - Experiment #1, (B) - Experiment #2. Data represents mean ± SE (n= 2).

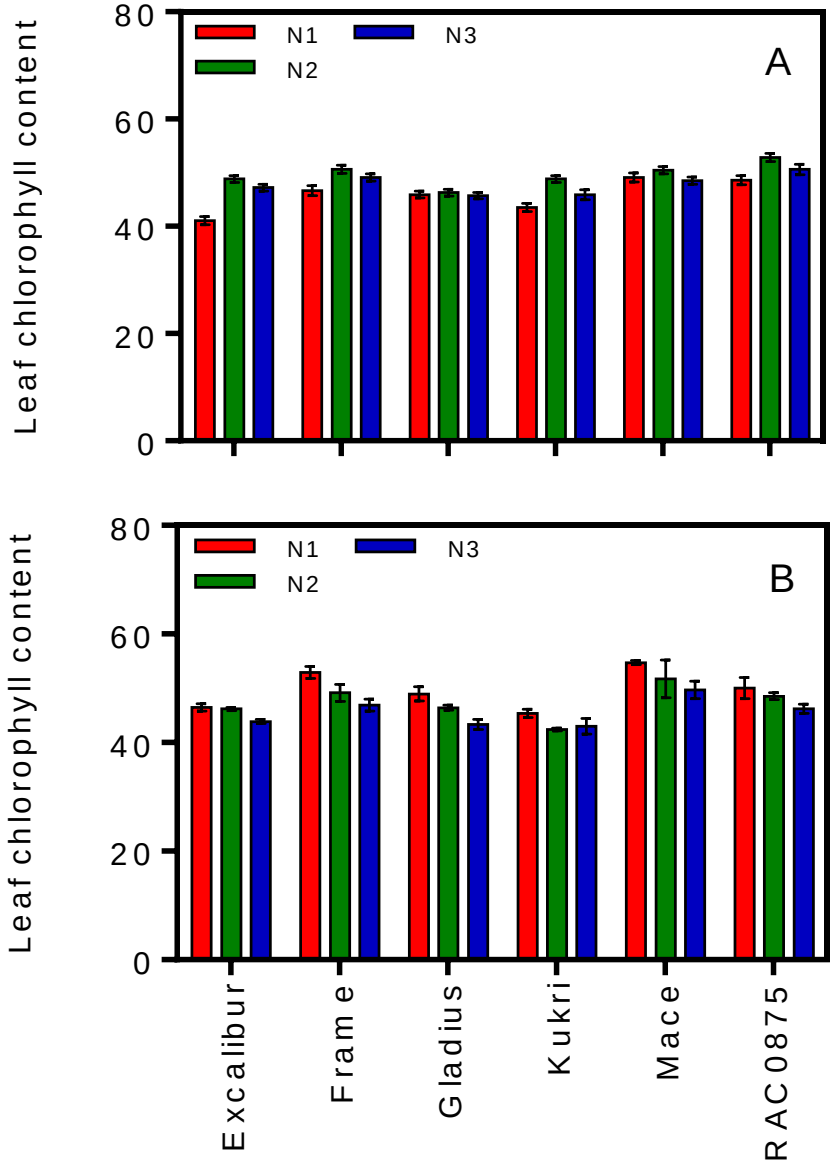

Figure 4.4. Leaf chlorophyll content in wheat varieties affected by N treatments. (A) - Experiment #1, (B) - Experiment #2. Data represents mean ±SE (n = 2).

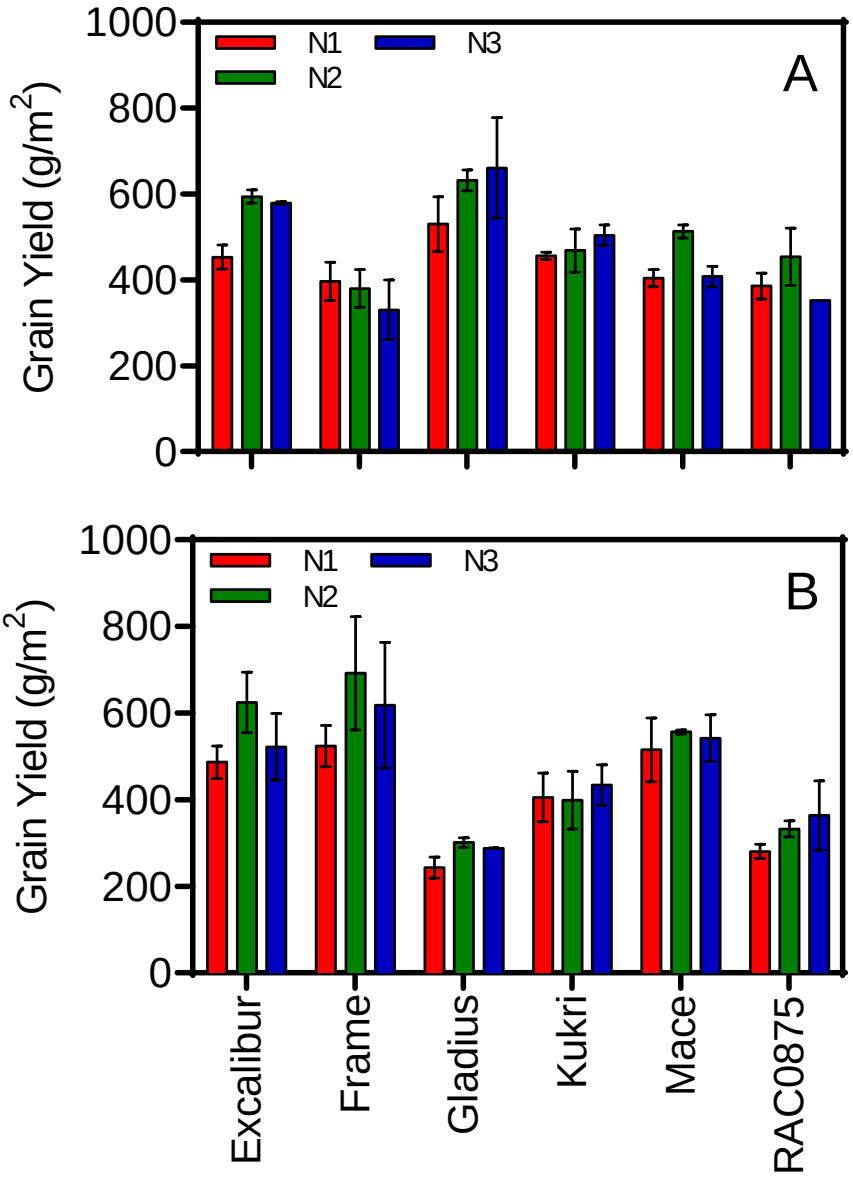

Figure 4.5. Grain yield / plant of wheat varieties affected by N treatments. (A) - Experiment #1, (B) - Experiment # 2. Data represents mean ± SE (n=2).

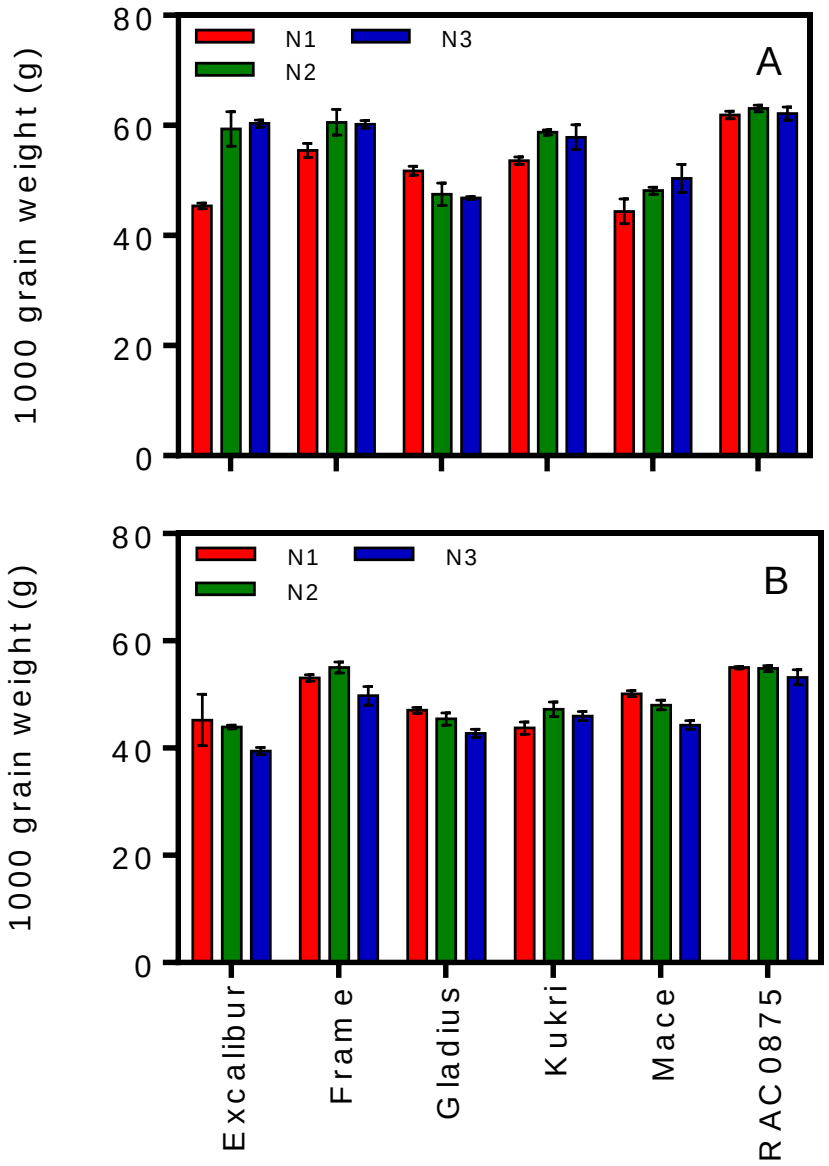

Figure 4.6. 1000 grain weight (g) in wheat varieties affected by N treatments. (A) - Experiment #1, (B) - Experiment #2. Data represents mean ± SE (n=2).

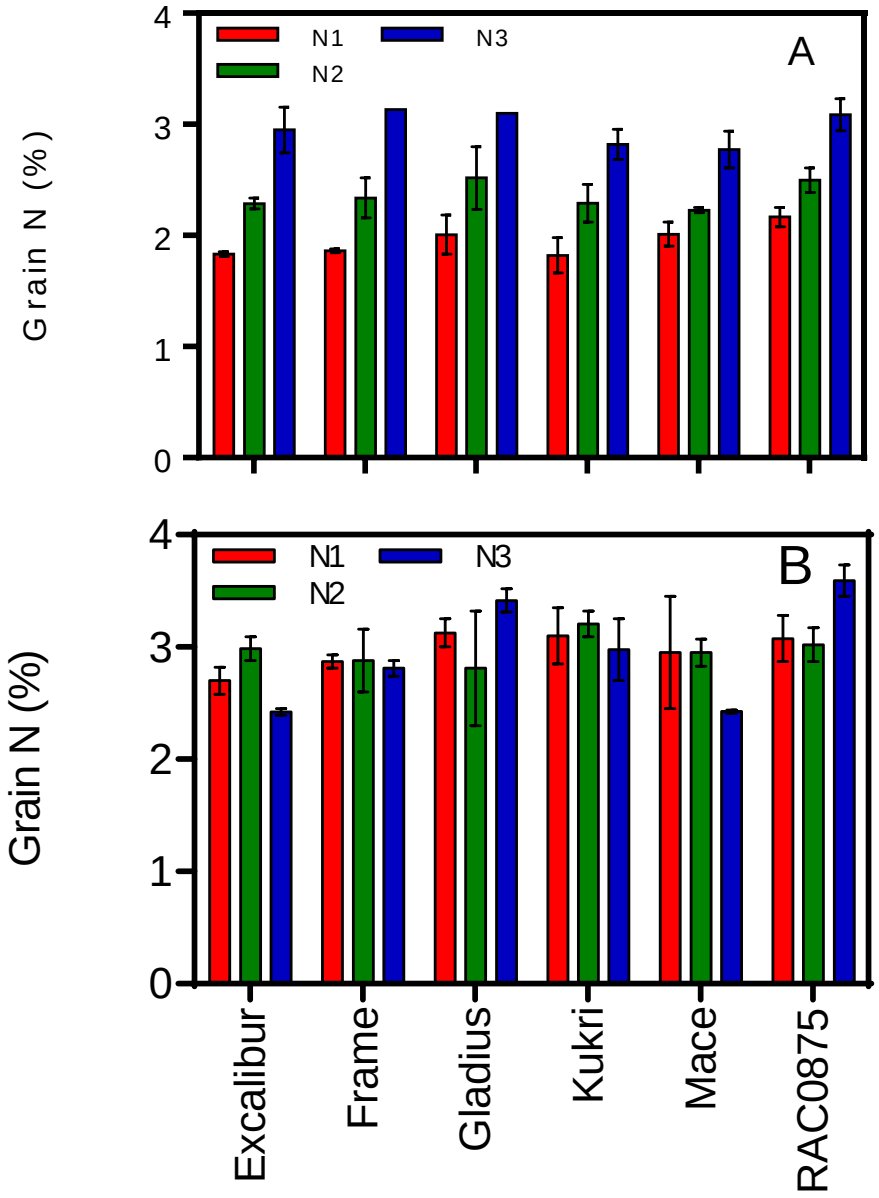

Figure 4.7. Grain N (%) in wheat varieties affected by N treatments. (A) - Experiment #1, (B) - Experiment #2. Data represents mean ± SE (n=2).

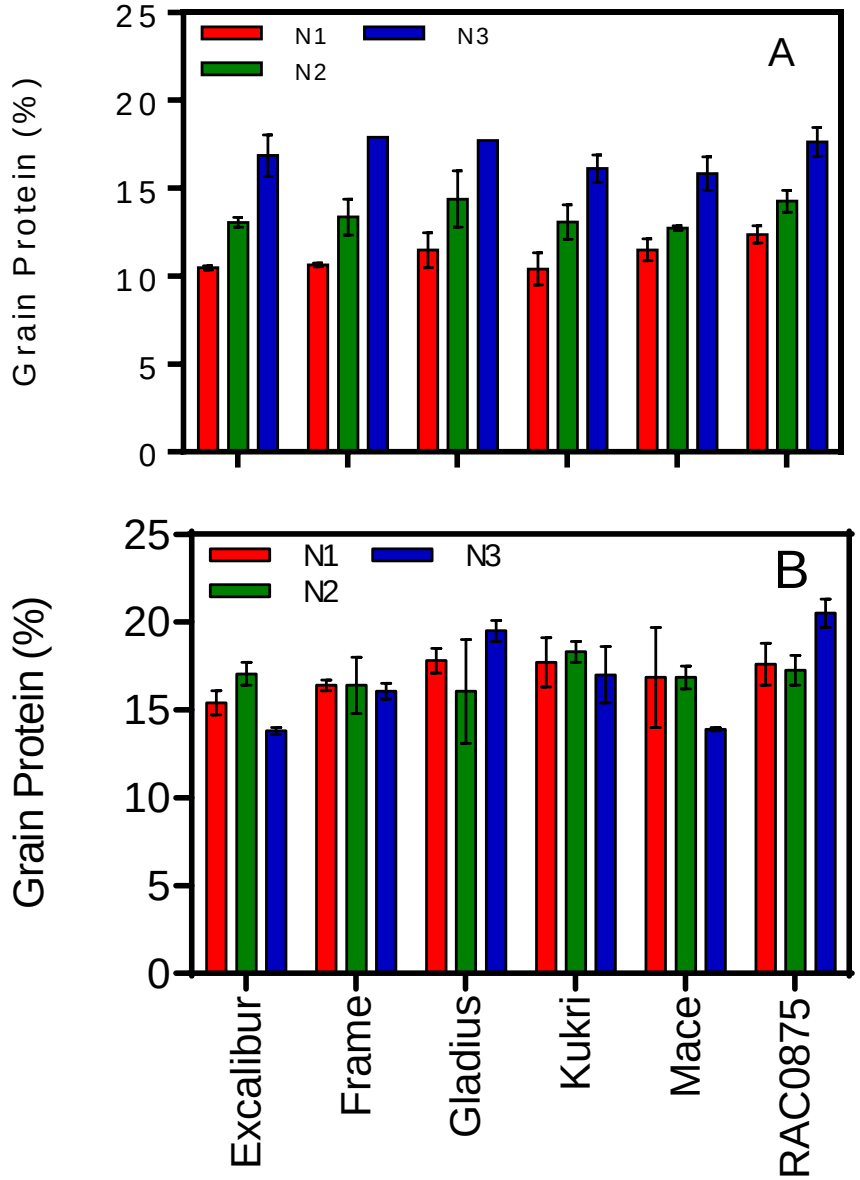

Figure 4.8. Grain protein % content in wheat varieties affected by N treatments. (A) - Experiment #1, (B) - Experiment #2. Data represents mean ± SE (n=2).

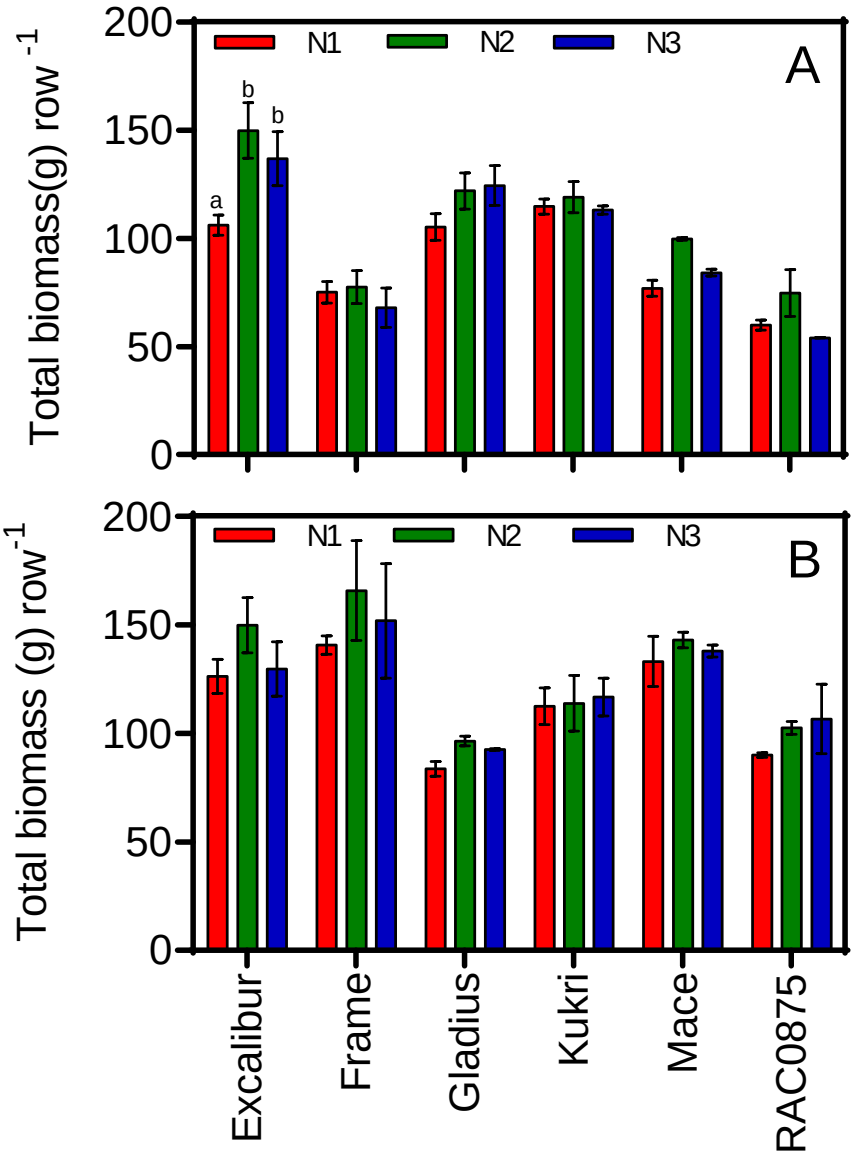

Figure 4.9. Total biomass (g/row) of wheat varieties affected by N treatments. (A) - Experiment #1, (B) - Experiment #2. Data represents mean ± SE (n=2).

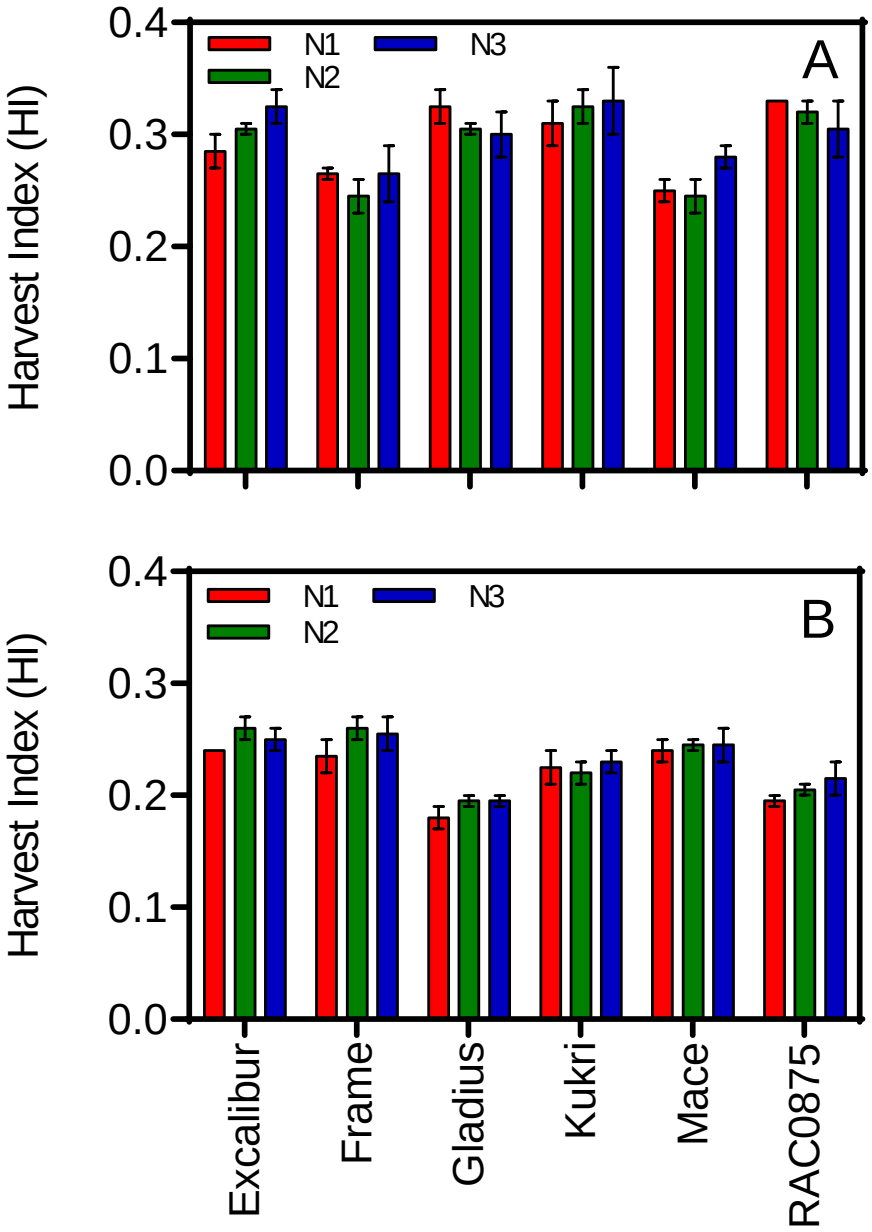

Figure 4.10. The effect of N treatments on Harvest Index (HI) in wheat varieties. (A) - Experiment #1, (B) - Experiment #2. Data represents mean ± SE (n=2).

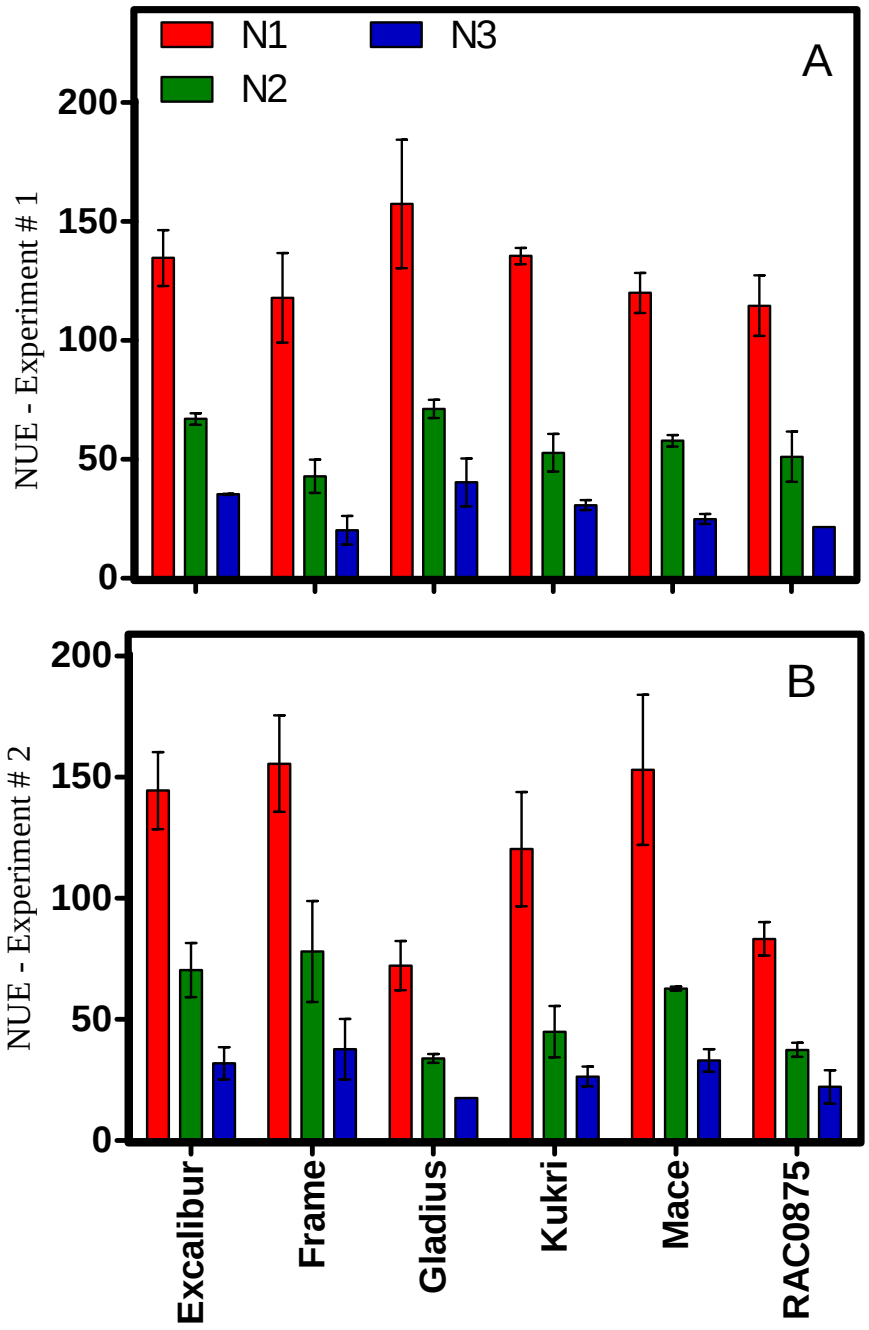

Figure 4. 11. (NUE) in wheat cultivars affected by N treatments. (A) - Experiment #1, (B) - Experiment #2. Data represents mean ± SE (n=2). NUE = grain yield (g/m^2) / available soil N (g/m^2).

CHAPTER 5

General Discussion

The selection of improved wheat germplasm is an on-going process to maintain yield and productivity. This objective is often linked to the availability of water and nutrients in the soil, where N is often a key macronutrient found in short supply across most agronomical important growing areas. The rising cost of N fertilisers, and the associated negative impact of their inefficient use on the environment, is driving future breeding programs to identify wheat varieties with improved nitrogen use efficiencies (NUE). In wheat, improved NUE must be accompanied with traits that maximise seed yield and if possible seed protein to ensure future crop productivity continues to meet expected yield and quality to feed a rapidly growing global population. To do this while utilising less N fertiliser inputs is a great challenge facing agronomist and plant scientists worldwide.

Australia's dryland agricultural regions are subjected to rainfall variability typified by extended wet and/or dry periods (Freebairn et al., 2006). Under these conditions, the ability of soils to store water and nutrients is an underlying component that helps to mediate and promote crop performance and yield. Farming practices, including nutrient management, have evolved to cope with the variability of Australia's dryland agricultural regions. For example, in northern cropping regions the use of fallows is common while in the southern and western regions, in-season adjustment of fertiliser inputs are common practice. Nitrogen management across most worldwide agricultural systems is built upon urea-based fertilizers (Bremner et al., 1995). This is primarily due to its high nitrogen content (46% nitrogen), solubility, inexpensive manufacturing costs, and the ability to store and transport (Gilbert et al., 2006; Prasad et al., 1998). A consequence of its use is the potential for N volatilization (and loss of N from the agricultural system) through soil-based hydrolytic processes that convert urea to ammonia (NH_3) and carbon dioxide (Bremner et al., 1995). Urea can also be lost through runoff and leaching with high rainfall and through soil-based immobilization and denitrification (Raun and Johnson 1999). The rate of NH_3 volatilisation is strongly influenced by soil moisture content when urea is surface applied. For example, rainfall (3–9 mm) within 5-9 days can result in losses of ammonium by 10 to 30%, while no rainfall within 6 days after surface urea application results in higher losses of ammonia, above 30% (Fox and Hoffman 1981). How plants adapt and grow in soils with applied N is a key component in developing

strategies that maximise N use in order to minimize N loss through traditional N depletion pressures discussed above.

In the current study, the responses of six wheat varieties to N fertilisation (Urea) were examined at three different locations in South Australia in 2010 and in two controlled glasshouse studies at the Waite campus at the University of Adelaide. The season was characterised as wet with 2010 growing season being the wettest year on record since 1992 (www.bom.gov.au). Unfortunately these conditions promoted N leaching and most likely movement of N downward from the root zone. As a result, I speculate the efficiency of N uptake across the wheat varieties may have been compromised and reduced yield and NUE may be evident in the data. Moreover, increased temperatures (18-24^{o}C) experienced at the later growing stages during grain filling may have also resulted in yield and or growth penalties. The optimum temperature for the growth and development of spring-wheat is around 20°C (Paulsen et al., 1994). When temperatures are high, the grain filling period often shortens and reduces yield (Housley & Ohm 1992; Wheeler et al., 1996; Moot et al., 1996; Altenbach et al., 2003; Gooding et al., 2003; Rahman et al., 2005). This may have been consequences of the environmental conditions experienced in 2010 across the three sites. Nonetheless, the data indicates that across the wheat varieties examined, location and soil conditions were strong determinants to yield and plant productivity with very little genetic differences evident in variety response to N supply during this trial.

Wheat N Field Trials (Mintaro, Pinnaroo and Tuckey)

Across the varieties there were a range of phenotypical differences to N treatment. From most of the measurements taken it was difficult to identify lines which consistently performed well across the varied field sites or equally when grown under controlled conditions in the glasshouse. The site of growth and the season of which the plants were grown (i.e. glasshouse experiments) were strong variables which dictated individual line performance to increasing N provision.

To evaluate the overall response between field sites to N treatment we pooled selected data sets across the six lines we tested. Grain yield and %N in the grain are two commercially relevant selection parameters which can be used to compare wheat lines for their ability to perform under varying growth conditions. In general, we found some differences between the three field sites in Grain %N in response to increasing N provision while Pinnaroo site showed highest response and Mintaro had moderate response but at Tuckey no response

(Figure 5.1, A). The grain N content was on average (1.39 %, 1.73 % and 1.54%) across each of the three sites and N treatments respectively.

There was a slight trend of increasing grain %N at both Mintaro and Pinnaroo, which is broadly in evidence across the individual lines. Grain yield at both Pinnaroo and Tuckey was vastly different from that of Mintaro achieving only ~50% of the Mintaro grain yield (Figure 5.1B). Surprisingly, the reduction in grain yield did not impact upon the final %N in the grain which was roughly equal to that of plants grown at the Mintaro site (as discussed above). The lower grain yield at Pinnaroo and Tuckey is most likely attributed to the overall decrease in shoot biomass and the capacity for sustained photosynthesis and grain production over the growing season (note: no shoot biomass measurements were taken for the Tuckey site). Although seed yield was significantly lower than Mintaro, lines grown at Tuckey did respond to increased N provision with a small increase in yield. A similar response was not observed at Pinnaroo, which may be related to the higher residual N content relative to that of Tuckey (Appendix 6). This result further highlights the strong impact of the poor growing conditions at Pinnaroo and Tuckey which overrides the potential impact of increased N provision.

In general, total biomass at Pinnaroo was approximately half that of the Mintaro trial, shoot biomass per harvested area did not vary across cultivars and N treatments of field trials; however there was a slight increase in total biomass with increased N levels only at Mintaro site (Fig 5. 2, B), This increase may be associated with larger leaves that stay green longer, tall stems and a large number of tillers surviving to maturity as was visual during the record of plant measurements, which was differed between the sites (soil residual N and soil texture). Moreover, HI was almost similar, ranged (0.34 – 0.39) in the field trials, no significant differences were observed with respect to N levels and different cultivars (Fig 5.2A). The differences in plant shoot biomass may be attributed to the plant height and greater leaf size as plants were responded to increased N levels. The finding is in agreement with observations made by (Khan et al., 2000).

Wheat N Glasshouse experiments

In the glasshouse studies, we found a strong response in grain %N to increasing N provision when plants were grown in the spring/summer season. However, autumn/winter grown plants demonstrated no N response but still achieved a uniform high %N level in the grain equal to the highest N treatment in the spring/summer grown plants (Figure 5.3A). Grain yield and

shoot biomass were found to be roughly similar across the N treatments and the two growing periods (5. 3B and 5.4B).

Total biomass differed between the two experiments due to the seasonal differences; more biomass was produced at autumn/winter than spring/summer season (Fig 5.4B). This was obvious when plant measurements were recorded; autumn/winter plants had larger leaves but were shorter than spring/ summer season plants which were taller with narrow leaves. This was may be due the differences in the length of the day between the two growing periods. However, no significant differences in HI were found between wheat cultivars and N treatments, but HI showed a general increase with elevated N levels. HI ranged from 0.24 to 0.32 in spring/ summer season and 0.18 to 26 in autumn/winter season (Fig 5.4A). The differences in HI of wheat varieties may be related to the differences in plant height that influence HI and /or related to the ability of genotypic responses to different N levels and available N in the soil for grain and shoot biomass production in which grain yield and HI is in an inverse relationship. The finding in this study may suggest that genetic improvement in yield was more associated with a genetic gain in biomass than in harvest index. As Shearman et al., (2005) also attributed yield improvements in varieties introduced in the UK between 1983 and 1995 to increased biomass rather than harvest index. Unfortunately the design of the glasshouse trials doesn't allow statistical testing of these observations and a note of caution needs to be stressed the data is preliminary until further experiments can be conducted to confirm the findings with appropriate replication and statistical anlayais.

Impact on wheat N use efficiency

In the present study we calculated NUE based on the yield response relative to applied N and residual N in the soil. As expected NUE was at its highest level in all genotypes at N1 and decreased sharply as N fertiliser was increased (N2-N4). As similar finding was reported by (Ortiz-Monasterio et al., 1997), where they calculated NUE only as grain production per unit of fertilizer N. In general, there was very little change in NUE response between varieties and growing locations (field or glasshouse). However, in greenhouse grown plants, NUE measured at N1 did vary in the autumn experiment where both Gladius and RAC0875 had a lower NUE compared to the other varieties tested. This result may be related to the number of heads per plant with these lines, which were significantly lower than most of the other lines across all N treatments.

In conclusion, results indicated that in general there was little variation in N responsiveness across the wheat lines tested with respect to NUE, grain yield, and %N in the harvested grain. The overriding variable that influenced the growth response of the lines was the combination of location and growing conditions. Poor growing sites including Pinnaroo and Tuckey were limiting the plants ability to respond to N provision and reach their ultimate growth potential. At Tuckey we observed the greatest response to N provision across the four N treatments. However the final grain yield achieved was only 50% of the fertile Mintaro site and approximately equal to the slightly improved Pinnaroo site. This result is not unexpected based on the growing sites, where soil N, water availability and temperature will have varied. The study also highlighted the significant differences in growth response between glasshouse and field based experiments. Although we observed different responses to applied N for grain %N across the two glasshouse experiments, the overall impact on yield and shoot biomass was negligible. In contrast the large fluctuations in yield potential observed across the three-field sites highlight the importance in site selection to dissect N-related growth and yield responses with future selection trials.

The results are indicating that Mace and Excalibur are superior cultivars and to a lesser extend Kukri due to improved higher yield and higher NUE value than the other three wheat cultivars. The finding indicates that medium N fertilization rate (75 – 100 kg N / ha) is to be the best recommendation in South Australian agriculture system to improve high yield and high grain quality with efficient use of nitrogen by plant in selecting new germplasm at the same time reducing the impact on environment and human concerns. The finding suggests that the recommendation of N-Fertilizer management with split application matches with plant demand during its life cycle is the best way to improve yield and enhanced NUE in crops than one application early in the season or at sowing time due to unpredictable time of rainfall. Moreover, heavy rainfall in winter causes to N insufficiency at tillering stage (Elliot et al., 1985).

However, the outcomes of one year of field experiments would likely not reflect the crop response information because of variation in growing season rainfall. Further research involving continued examination in the field and glasshouse is an important approach to better understand the N responsiveness of these varieties and their suitability as parental lines to analyse genetic diversity for NUE traits.

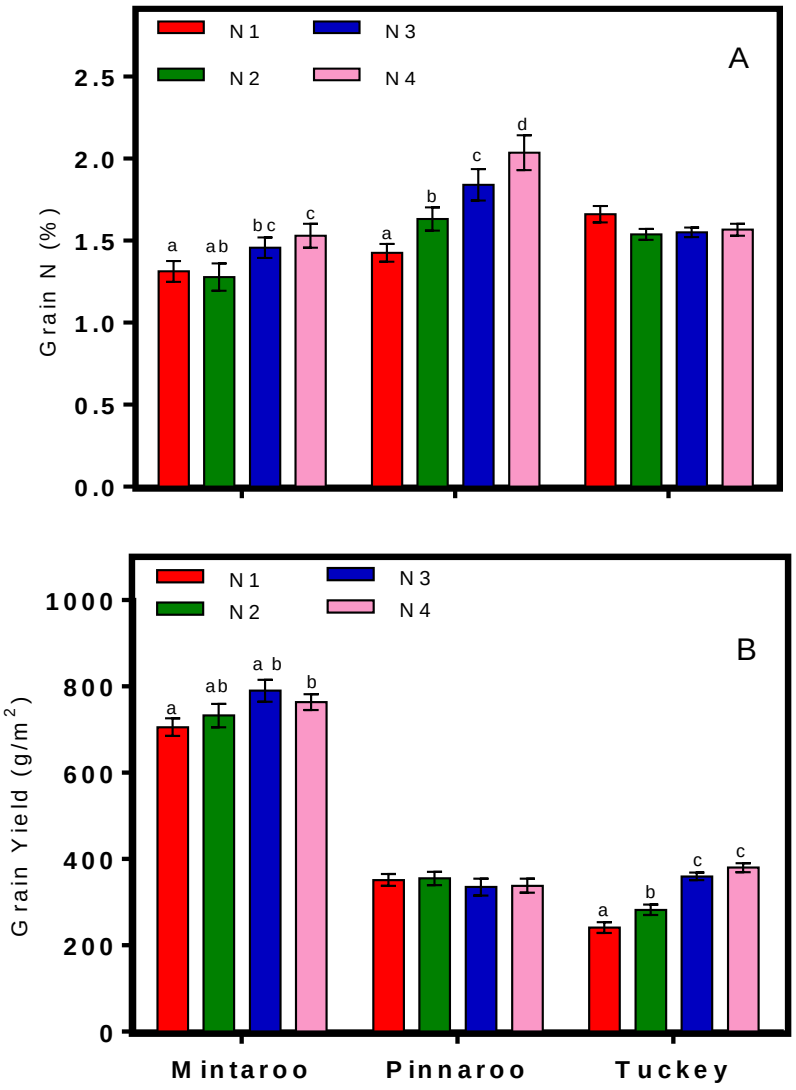

Figure 5.1. Wheat cultivars response to different N treatments at the three sites: (A) - grain N % content, (B) - grain yield. Data represents mean ± SE (n=3).

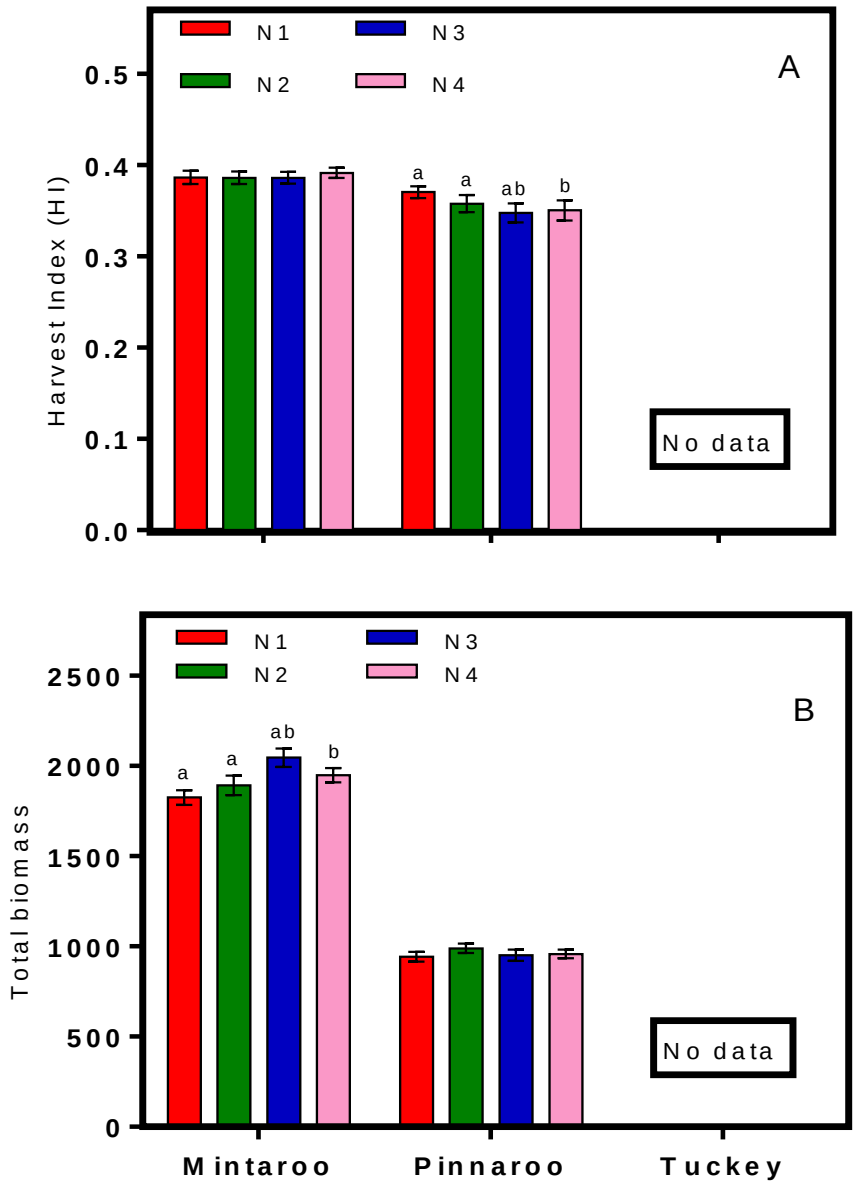

Figure 5.2. Wheat Cultivars response to different N treatments at the three sites: (A) - Harvest Index (HI), (B) - Total biomass. Data represents mean ± SE (n=3).

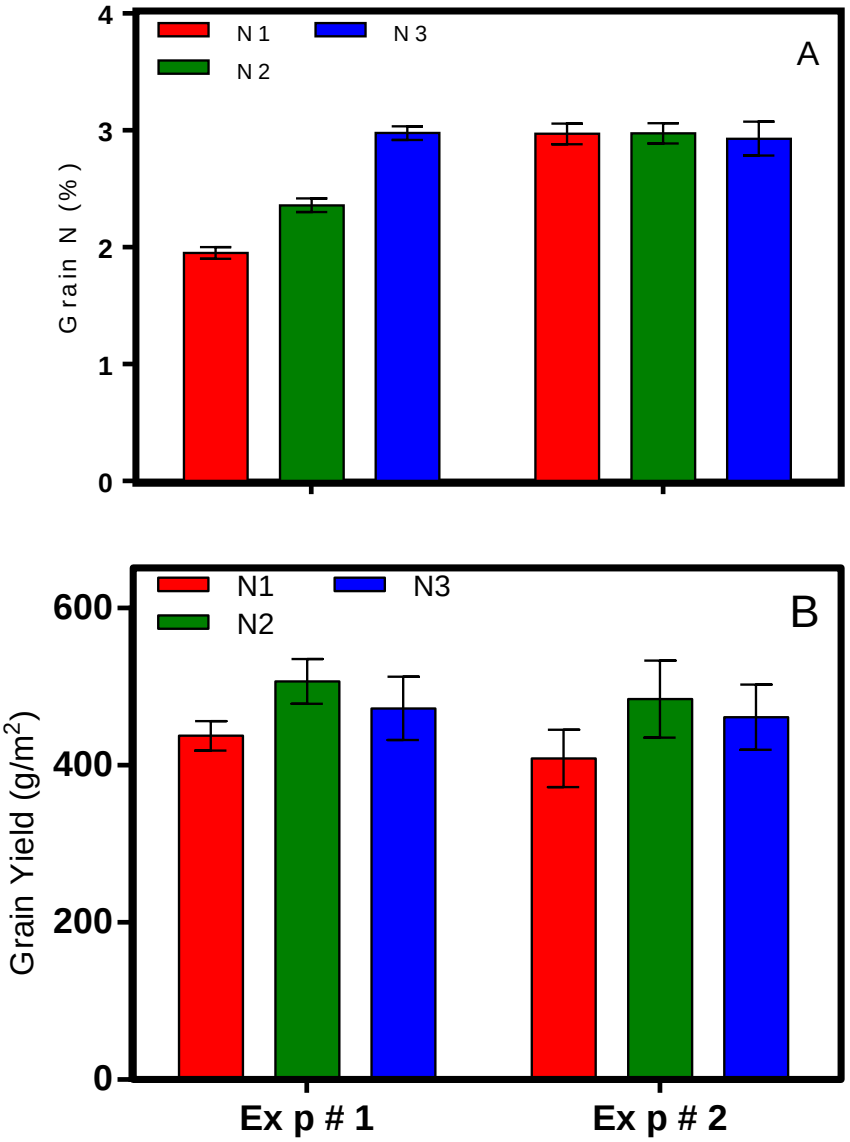

Figure 5.3. Wheat Cultivars response to different N treatments in the glasshouse experiments: (A) - grains N % content, (B) - grain yield**.** Data represents mean ± SE (n=2).

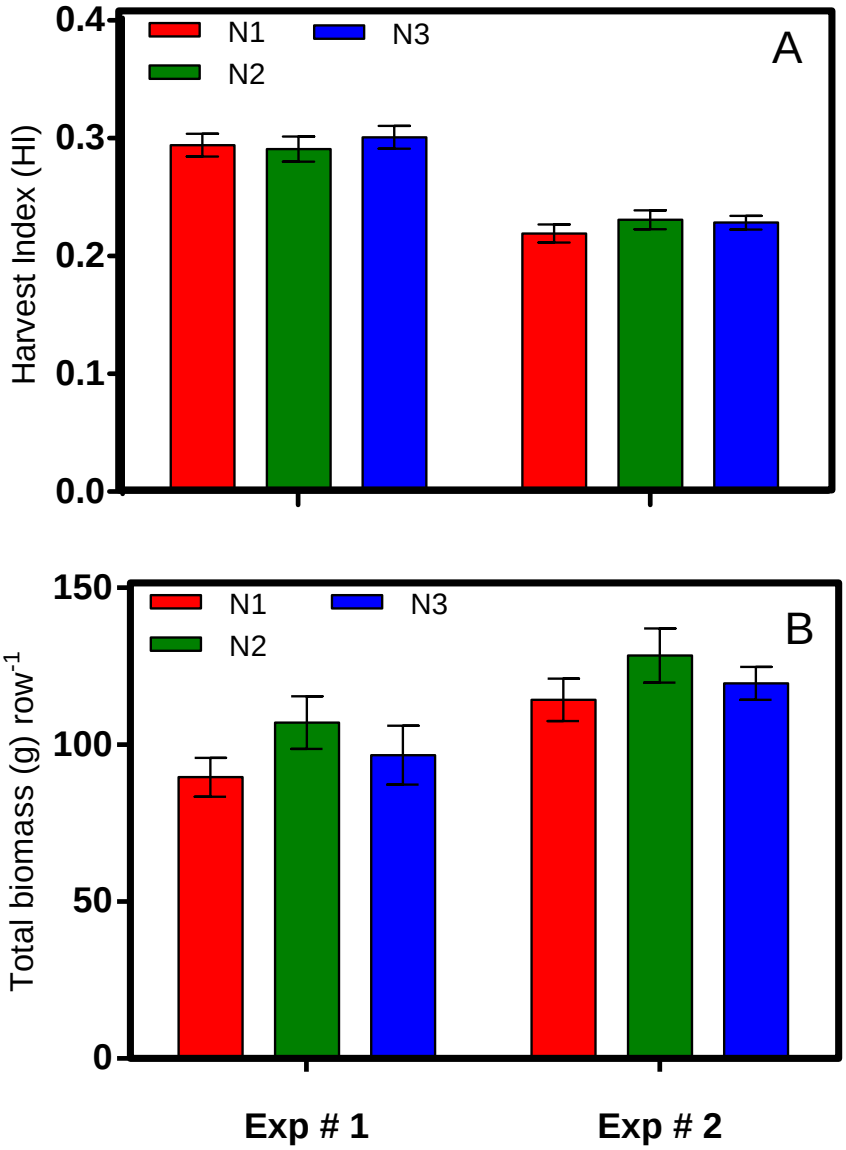

Figure 5.4. Wheat Cultivars response to different N treatments in the glasshouse experiments: (A) - Harvest Index (HI), (B) - Total biomass**.** Data represents mean ± SE (n=2).

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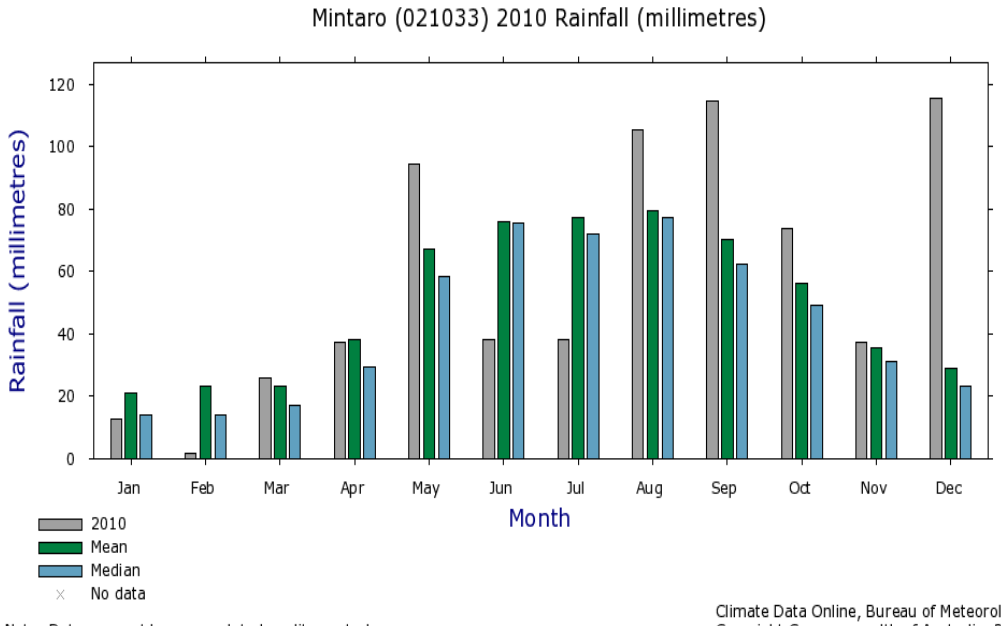
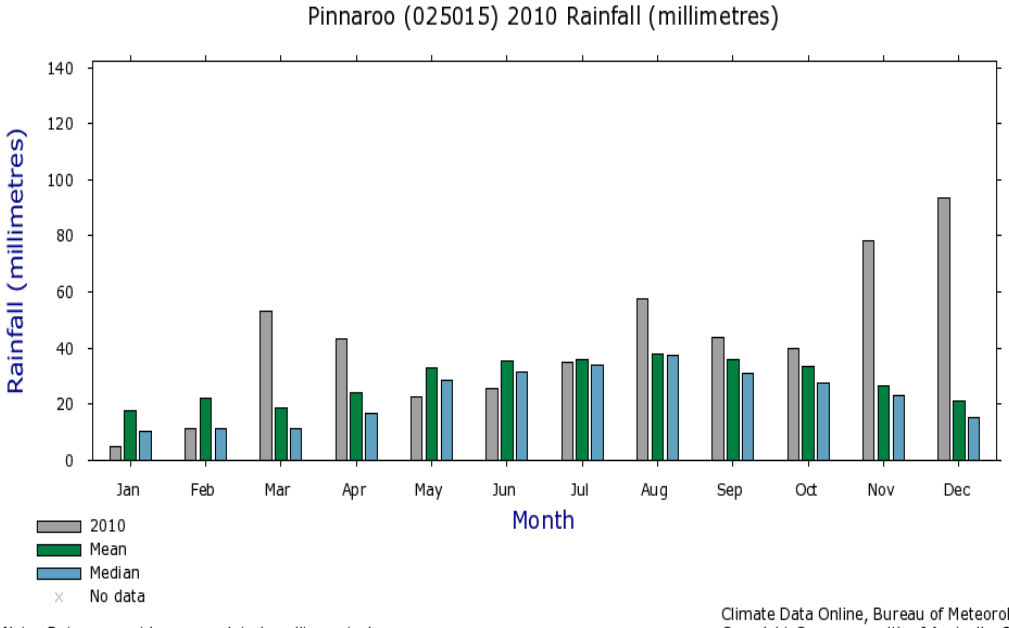
APPENDIXES

Appendix 1: Mintaro rainfall (mm) 2010 growing season



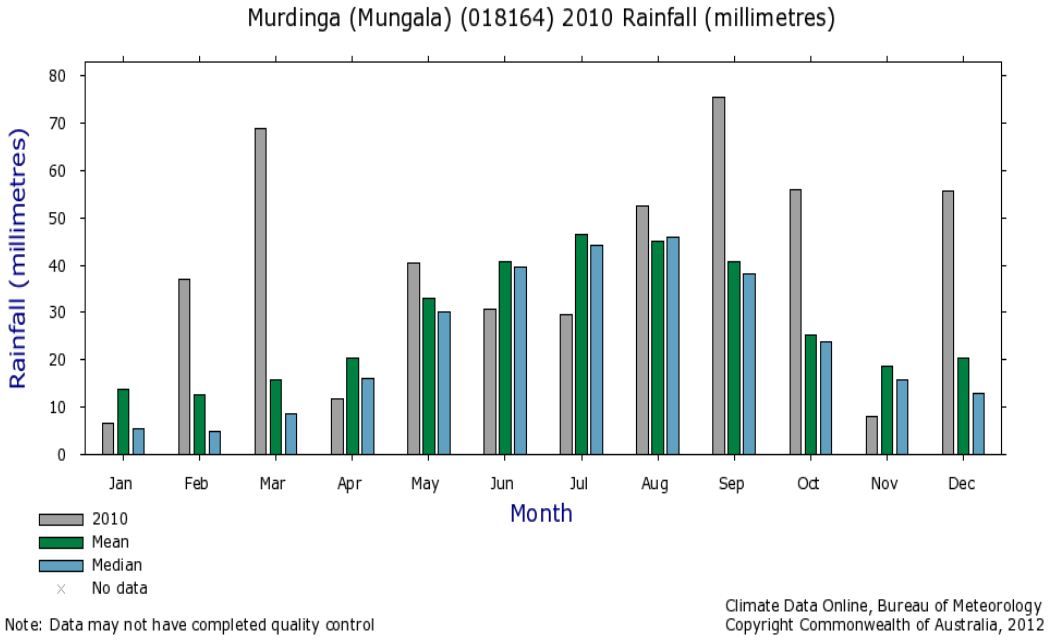
Note: Data may not have completed quality control

Appendix 2: Pinnaroo rainfall (mm) 2010 growing season



Note: Data may not have completed quality control

Appendix 3: Tuckey rainfall (mm) 2010 growing season



Appendix 4: Field trials analysis using two-way general ANOVA (Genstat)

A – Mintaro

Analysis of variance

Variate: Height

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	40.33	20.17	1.14	
Block.Cultivar stratum					
Cultivar	5	840.67	168.13	9.47	0.001
Residual	10	177.50	17.75	0.97	
Block.Cultivar.Nitrogen stratum					
Nitrogen	3	13.39	4.46	0.24	0.865
Cultivar.Nitrogen	15	103.44	6.90	0.38	0.977
Residual	36	658.17	18.28		
Total	71	1833.50			

Fisher's protected least significant difference test

Cultivar

	Mean	
G	76.00	a
RAC0875	78.50	ab
F	79.58	ab
M	82.00	bc
E	84.75	c
K	85.67	c

B- Tuckey

Analysis of variance

Variate: Height

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	56.08	28.04	1.84	
Block.Cultivar stratum					
Cultivar	5	730.83	146.17	9.58	0.001
Residual	10	152.58	15.26	0.62	
Block.Cultivar.Nitrogen stratum					
Nitrogen	3	1429.83	476.61	19.22	<.001
Cultivar.Nitrogen	15	291.50	19.43	0.78	0.686
Residual	36	892.67	24.80		
Total	71	3553.50			

Fisher's protected least significant difference test

Cultivar

	Mean	
G	78.25	a
F	79.17	a
RAC0875	80.58	ab
K	83.58	bc
E	84.67	cd
M	87.25	d

Fisher's protected least significant difference test

Nitrogen

	Mean	
0	75.44	a
50	81.50	b
100	84.61	bc
150	87.44	c

A-Mintaro

Analysis of variance

Variate: Head

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	35355.	17677.	4.46	
Block.Cultivar stratum					
Cultivar	5	79389.	15878.	4.00	0.030
Residual	10	39655.	3965.	0.95	
Block.Cultivar.Nitrogen stratum					
Nitrogen	3	14733.	4911.	1.17	0.334
Cultivar.Nitrogen	15	88144.	5876.	1.40	0.199
Residual	36	150947.	4193.		
Total	71	408223.			

Fisher's protected least significant difference test

Cultivar

	Mean	
RAC0875	483.3	a
K	525.8	ab
E	529.0	ab
F	546.0	b
G	576.4	b
M	581.5	b

B- Pinnaroo

Analysis of variance

Variate: Head

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	9117.	4558.	1.47	
Block.Cultivar stratum					
Cultivar	5	48391.	9678.	3.12	0.059
Residual	10	31054.	3105.	2.38	
Block.Cultivar.Nitrogen stratum					
Nitrogen	3	1227.	409.	0.31	0.815
Cultivar.Nitrogen	15	36932.	2462.	1.89	0.059
Residual	36	46922.	1303.		
Total 71	173642.				

A-Mintaro

Analysis of variance

Variate: Yield

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	84660.	42330.	9.71	
Block.Cultivar stratum					
Cultivar	5	165984.	33197.	7.62	0.003
Residual	10	43575.	4357.	0.59	
Block.Cultivar.Nitrogen stratum					
Nitrogen	3	72605.	24202.	3.25	0.033
Cultivar.Nitrogen	15	94488.	6299.	0.85	0.623
Residual	36	267721.	7437.		
Total	71	729033.			

Fisher's protected least significant difference test

Cultivar

	Mean	
F	687.2	a
K	694.0	a
RAC0875	741.3	ab
E	762.8	b
G	777.2	bc
M	826.1	c

Fisher's protected least significant difference test

Nitrogen

	Mean	
0	705.7	a
50	732.9	ab
150	763.8	ab
100	790.0	b

B- Pinnaroo

Analysis of variance

Variate: Yield

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	48821.	24411.	5.36	
Block.Cultivar stratum					
Cultivar	5	77718.	15544.	3.41	0.047
Residual	10	45572.	4557.	1.62	
Block.Cultivar.Nitrogen stratum					
Nitrogen	3	5214.	1738.	0.62	0.607
Cultivar.Nitrogen	15	53060.	3537.	1.26	0.276
Residual	36	100991.	2805.		
Total	71	331377.			

Fisher's protected least significant difference test

Cultivar

	Mean	
F	308.8	a
K	319.4	a
G	332.3	a
RAC0875	342.8	a
E	356.1	ab
M	410.0	b

Fisher's protected least significant difference test

Nitrogen

	Mean	
0	1.426	a
50	1.632	b
100	1.841	c
150	2.036	d

C- Tuckey

Analysis of variance

Variate: Yield

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	14787.	7394.	4.74	
Block.Cultivar stratum					
Cultivar	5	43667.	8733.	5.60	0.010
Residual	10	15586.	1559.	0.98	
Block.Cultivar.Nitrogen stratum					
Nitrogen	3	230108.	76703.	48.00	<.001
Cultivar.Nitrogen	15	17654.	1177.	0.74	0.733
Residual	36	57523.	1598.		
Total	71	379326.			

Fisher's protected least significant difference test

Cultivar

	Mean	
G	282.0	a
K	290.9	ab
F	318.8	b
RAC0875	320.2	b
E	325.7	bc
M	357.7	c

Fisher's protected least significant difference test

Nitrogen

	Mean	
0	241.2	a
50	282.2	b
100	359.9	c
150	380.2	c

A-Mintaro

Analysis of variance

Variate: thgw

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	22.503	11.252	1.57	
Block.Cultivar stratum					
Cultivar	5	518.093	103.619	14.48	<.001
Residual	10	71.583	7.158	0.85	
Block.Cultivar.Nitrogen stratum					
Nitrogen	3	87.147	29.049	3.45	0.027
Cultivar.Nitrogen	15	138.760	9.251	1.10	0.392
Residual	36	303.353	8.426		
Total	71	1141.440			

Fisher's protected least significant difference test

Nitrogen

	Mean	
0	46.71	a
150	46.71	a
100	48.67	ab
50	49.11	b

Fisher's protected least significant difference test

Cultivar

	Mean	
M	44.62	a
E	45.18	ab
K	47.15	bc
G	47.77	c
F	49.50	c
RAC0875	52.58	d

B- Pinnaroo

Analysis of variance

Variate: thgw

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	71.929	35.964	2.50	
Block.Cultivar stratum					
Cultivar	5	680.119	136.024	9.45	0.002
Residual	10	143.938	14.394	1.45	
Block.Cultivar.Nitrogen stratum					
Nitrogen	3	240.492	80.164	8.09	<.001
Cultivar.Nitrogen	15	151.182	10.079	1.02	0.460
Residual	36	356.567	9.905		
Total	71	1644.226			

Fisher's protected least significant difference test

Cultivar

	Mean	
K	39.00	a
E	39.68	ab
M	39.77	ab
G	42.76	bc
RAC0875	45.49	cd
F	47.01	d

C- Tuckey

Analysis of variance

Variate: thgw

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	1.123	0.562	0.09	
Block.Cultivar stratum					
Cultivar	5	224.453	44.891	7.54	0.004
Residual	10	59.523	5.952	1.46	
Block.Cultivar.Nitrogen stratum					
Nitrogen	3	39.720	13.240	3.25	0.033
Cultivar.Nitrogen	15	111.053	7.404	1.82	0.071
Residual	36	146.527	4.070		
Total	71	582.400			

Fisher's protected least significant difference test

Cultivar

	Mean	
K	43.42	a
E	43.88	ab
F	44.85	ab
G	45.40	ab
M	46.02	b
RAC0875	48.83	c

Fisher's protected least significant difference test

Nitrogen

	Mean	
150	44.36	a
100	45.17	ab
50	45.69	ab
0	46.39	b

A-Mintaro

Analysis of variance

Variate: grain N

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	1.75859	0.87929	12.10	
Block.Cultivar stratum					
Cultivar	5	0.72264	0.14453	1.99	0.166
Residual	10	0.72646	0.07265	1.17	
Block.Cultivar.Nitrogen stratum					
Nitrogen	3	0.76046	0.25349	4.09	0.013
Cultivar.Nitrogen	15	0.74307	0.04954	0.80	0.670
Residual	36	2.22855	0.06190		
Total	71	6.93977			

B- Pinnaroo

Analysis of variance

Variate: grain N

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	0.52820	0.26410	3.71	
Block.Cultivar stratum					
Cultivar	5	3.37062	0.67412	9.48	0.001
Residual	10	0.71141	0.07114	0.90	

Block.Cultivar.Nitrogen stratum					
Nitrogen	3	3.74505	1.24835	15.76	<.001
Cultivar.Nitrogen	15	1.19894	0.07993	1.01	0.468
Residual	36	2.85146	0.07921		
Total	71	12.40568			

Fisher's protected least significant difference test

Cultivar

	Mean	
M	1.331	a
K	1.646	b
E	1.671	b
G	1.866	bc
RAC0875	1.915	c
F	1.973	c

Fisher's protected least significant difference test

Nitrogen

	Mean	
150	40.42	a
50	41.20	a
100	42.29	a
0	45.23	b

C- Tuckey

Analysis of variance

Variate: grainN

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	0.02183	0.01091	0.28	
Block.Cultivar stratum					
Cultivar	5	0.29201	0.05840	1.50	0.272
Residual	10	0.38853	0.03885	1.90	
Block.Cultivar.Nitrogen stratum					
Nitrogen	3	0.16761	0.05587	2.73	0.058

Cultivar.Nitrogen	15	0.32178	0.02145	1.05	0.433
Residual	36	0.73618	0.02045		
Total	71	1.92794			

A-Mintaro

Analysis of variance

Variate: Biomass

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	282036.	141018.	5.16	
Block.Cultivar stratum					
Cultivar	5	322354.	64471.	2.36	0.116
Residual	10	273426.	27343.	0.66	
Block.Cultivar.Nitrogen stratum					
Nitrogen	3	468731.	156244.	3.78	0.019
Cultivar.Nitrogen	15	349650.	23310.	0.56	0.883
Residual	36	1488346.	41343.		
Total	71	3184542.			

Fisher's protected least significant difference test

Nitrogen

	Mean	
0	1826	a
50	1893	a
150	1949	ab
100	2046	b

B- Pinnaroo

Analysis of variance

Variate: Biomass

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	88440.	44220.	2.31	
Block.Cultivar stratum					

Cultivar	5	87374.	17475.	0.91	0.511
Residual	10	191587.	19159.	1.93	
Block.Cultivar.Nitrogen stratum					
Nitrogen	3	22484.	7495.	0.76	0.527
Cultivar.Nitrogen	15	189400.	12627.	1.27	0.269
Residual	36	357270.	9924.		
Total	71	936556.			

A-Mintaro

Analysis of variance

Variate: HI

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	0.0019528	0.0009764	2.49	
Block.Cultivar stratum					
Cultivar	5	0.0151569	0.0030314	7.75	0.003
Residual	10	0.0039139	0.0003914	0.62	
Block.Cultivar.Nitrogen stratum					
Nitrogen	3	0.0003931	0.0001310	0.21	0.891
Cultivar.Nitrogen	15	0.0076153	0.0005077	0.80	0.671
Residual	36	0.0228667	0.0006352		
Total	71	0.0518986			

Fisher's protected least significant difference test

Cultivar

	Mean	
F	0.3692	a
RAC0875	0.3733	a
K	0.3792	ab
E	0.3950	bc
G	0.4017	c
M	0.4075	c

B- Pinnaroo

Analysis of variance

Variate: H1

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	0.0176253	0.0088126	4.99	
Block.Cultivar stratum					
Cultivar	5	0.0371837	0.0074367	4.21	0.025
Residual	10	0.0176648	0.0017665	2.72	
Block.Cultivar.Nitrogen stratum					
Nitrogen	3	0.0057291	0.0019097	2.94	0.046
Cultivar.Nitrogen	15	0.0120704	0.0008047	1.24	0.290
Residual	36	0.0233889	0.0006497		
Total	71	0.1136622			

Fisher's protected least significant difference test

Nitrogen

	Mean	
100	0.3489	a
150	0.3506	a
50	0.3574	ab
0	0.3716	b

Fisher's protected least significant difference test

Cultivar

	Mean	
F	0.3268	a
K	0.3352	ab
RAC0875	0.3555	ab
G	0.3617	abc
E	0.3666	bc
M	0.3969	c

A-Mintaro

Analysis of variance

Variate: NUE

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	1307.94	653.97	9.37	
Block.Cultivar stratum					
Cultivar	5	2148.86	429.77	6.16	0.007
Residual	10	698.06	69.81	0.72	
Block.Cultivar.Nitrogen stratum					
Nitrogen	3	33467.89	11155.96	114.27	<.001
Cultivar.Nitrogen	15	1555.96	103.73	1.06	0.421
Residual	36	3514.46	97.62		
Total	71	42693.17			

Fisher's protected least significant difference test

Cultivar

	Mean	
K	78.50	a
F	79.25	ab
RAC0875	84.08	abc
E	86.28	bc
G	87.19	c
M	94.82	d

Fisher's protected least significant difference test

Nitrogen

	Mean	
50	1.278	a
0	1.313	ab
100	1.457	bc
150	1.529	c

B- Pinnaroo

Analysis of variance

Variate: NUE

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	940.71	470.36	6.45	
Block.Cultivar stratum					
Cultivar	5	1182.35	236.47	3.24	0.053
Residual	10	728.87	72.89	1.35	
Block.Cultivar.Nitrogen stratum					
Nitrogen	3	17721.54	5907.18	109.30	<.001
Cultivar.Nitrogen	15	614.99	41.00	0.76	0.711
Residual	36	1945.64	54.05		
Total	71	23134.09			

Fisher's protected least significant difference test

Nitrogen

	Mean	
150	28.21	a
100	34.62	b
50	48.11	c
0	69.19	d

C- Tuckey

Analysis of variance

Variate: NUE

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	556.88	278.44	2.55	
Block.Cultivar stratum					
Cultivar	5	1463.30	292.66	2.68	0.087
Residual	10	1093.07	109.31	1.26	
Block.Cultivar.Nitrogen stratum					
Nitrogen	3	15284.54	5094.85	58.95	<.001
Cultivar.Nitrogen	15	1078.61	71.91	0.83	0.638
Residual	36	3111.30	86.42		
Total	71	22587.69			

Fisher's protected least significant difference test

Nitrogen

	Mean	
150	37.91	a
100	46.55	b
50	51.97	b
0	77.07	c

Appendix 5: Field trials, soil physical and chemical characteristics

The initial soil analysis report (2010)				
	Name	**Pinnaroo**	**Mintaro**	**Tuckey**
Depth		0-10 cm	0-10 cm	0-10 cm
Colour		GR	BR	BRGR
Gravel	%	0	0	0
Texture		3.5	3.5	1.5
Ammonium Nitrogen	mg/Kg	3	7	3
Nitrate Nitrogen	mg/K	25	28	10
Phosphorus Colwell	mg/Kg	48	67	42
Potassium Colwell	mg/Kg	724	715	431
Sulphur	mg/Kg	8.35	22.40	422
Organic Carbon	%	1.18	1.99	0.96
Conductivity	dS/m	0. 217	0.318	0.183
pH level ($CaCl_2$)	pH	7.77	6.88	7.47
pH level (H_2O)	pH	8.57	7.42	8.13
DTPA Copper	mg/Kg	0.51	1.70	0.37
DTPA Iron	mg/Kg	15.58	48.42	68.31
DTPA Manganese	mg/Kg	7.50	28.85	5.70
DTPA Zinc	mg/Kg	1.45	1.60	2.47
Exc. Aluminium	meq/100g	<0.001	<0.001	<0.0

Appendix 6: Soil available N at the three sites and glasshouse experiments

Sites	**NO^{3-} -N**	**NH^{4+} -N**	**Total available N**
Mintaro	36.4	9.1	45.5 kg/ha
Pinnaroo	32.5	3.9	36.4 kg/ha
Tuckey	13	3.9	16.9 kg/ha
GH-experiments	13	0.65	13.65 kg/ha

Appendix 7: Soil texture and bulk density for different soil types

Soil texture	**Bulk density (g/cm3)**
Coarse sand	1.3 – 1.8
Fine sand	1.3
Light sandy clay loam	1.3 – 1.6
Loam	1.1 – 1.4
Sandy clay loam	1.3 – 1.6
Clay loam	1.3 – 1.6
Clay	1.3 – 1.5
Self mulching clay	1.2 – 1.3

Appendix 8: Glasshouse experiments, soil physical and chemical characteristics

Name	Unit (mg/kg)	Name	Unit (mg/kg)
pH (1:5 soil:water)	8.5	Mo	<10
pH (0.01M $CaCl_2$	7.9	Na	57.9
NH4-N	0.5	Ni	7.38
NO3-N	10	P	158
Al	13500	Pb	<10
As	<10	S	51.3
B	10.3	Sb	<10
Ca	1720	Se	<10
Cd	<10	Zn	10.9
Co	<10		
Cr	13.3		
Cu	<10		

Appendix 9: List of 24 wheat cultivars used at the three sites by (AGT) in (2010), including selected six wheat varieties used in this project

Wheat cultivars	**Wheat cultivars**
AGT-KATANA	MACE
AXE	RAC0875
CATALINA	RAC1669R
CORRELL	RAC1671R
DERRIMUT	RAC1412
DRYSDALE	RAC1569
ESPADA	RAC1683
EXCALIBUR	WESTONIA
FRAME	WYALKATCHEM
GLADIUS	WAGT104
JANZ	YITPI
KUKRI	YOUNG

Appendix10: Micronutrient supplied in the glasshouse experiment for adjusting nutrient deficiency

Micronutrients	**1000 x Stock**	**mg l^{-1}**
H_3BO_3	25 mM	1.546 g
$ZnSO_4$	2 µM	575 mg
$MnSO_4$	2 mM	338 mg
$CuSO_4$	0.5 mM	125 mg
(H_2MoO_4) $(NH_4)_6M0_7O_{24}$	0.5 mM	618 mg