

**University of São Paulo
“Luiz de Queiroz” College of Agriculture**

Nitrogen impacts on maize yield and nutrient use efficiency in contrasting stress environments

Adilson Nunes da Silva

Thesis presented to obtain the degree of Doctor in
Science. Area: Crop Science

**Piracicaba
2015**

Adilson Nunes da Silva
Agronomist

Nitrogen impacts on maize yield and nutrient use efficiency in contrasting stress environments

versão revisada de acordo com a resolução CoPGr 6018 de 2011

Advisor:
Prof. Dr. **KLAUS REICHARDT**

Thesis presented to obtain the degree of Doctor in
Science. Area: Crop Science

Piracicaba
2015

**Dados Internacionais de Catalogação na Publicação
DIVISÃO DE BIBLIOTECA - DIBD/ESALQ/USP**

Silva, Adilson Nunes da

Nitrogen impacts on maize yield and nutrient use efficiency in contrasting stress environments / Adilson Nunes da Silva. - - versão revisada de acordo com a resolução CoPGr 6018 de 2011. - - Piracicaba, 2015.

178 p. : il.

Tese (Doutorado) - - Escola Superior de Agricultura "Luiz de Queiroz".

1. Tempo e doses de nitrogênio 2. Densidade de plantio 3. Fotossíntese 4. Genótipos tolerantes ao déficit hídrico I. Título

CDD 633.15
S586n

“Permitida a cópia total ou parcial deste documento, desde que citada a fonte – O autor”

DEDICATION

To my parents Antonio Nunes and Nadir Olivia, who supported me with all the love that they had to offer.

ACKNOWLEDGMENT

I am so grateful for all the support I have received while working on my dissertation. First, none of this would have been possible without God's love and the endless possibilities He creates. Next, my family's support has been endless. My parents, Antonio Nunes and Nadir Olivia, and my brother and sister, André and Adriana, have always been there for me with support, love, and encouragement. Their presence in my life has made this possible.

My advisor, Dr. Klaus Reichardt, has also been instrumental in my success. I cannot thank him enough for his friendship, mentoring, and patience. In addition, my committee has been very helpful. Specifically, Dr. Tony J. Vyn, his trust in me, and the opportunities he provided for me at Purdue University helped make my dissertation possible.

Along these lines, I would like to thank Luiz de Queiroz College of Agriculture, University of Sao Paulo (USP) and Purdue University. The departments of Crop Science and Agronomy both provided an excellent education and numerous opportunities. Special thanks needs to be given to two secretaries at USP, Luciane Aparecida Toledo and Maria Solizete Silva, for answering numerous questions, providing moral support, and their continued professionalism. At Purdue University, technicians Alicia West and Terry West taught me a great deal about the equipment and methodologies in agronomy, thank you.

To Professor Paulo Cesar Ocheuze Trivelin and the Stabel Isotope Laboratory at CENA/USP, I would like to thank for the access to the research area and the use of laboratory equipment for my Brazilian research.

I would also like to thank Professors Durval Dourado Neto, Ricardo Kluge, Carlos Guilherme Silveira Pedreira, José Laércio Favarin, Ricardo Antunes de Azevedo, Simone Mello, Paulo Roberto de Camargo e Castro, Pedro Christoffoleti, and Ricardo Ferraz de Oliveira, for access and use of laboratory equipment and the questions and help they were willing to provide. The students, both graduate and undergraduate, who collected and analyzed samples with me in the field and in the laboratories, were immensely helpful.

The continued financial support I received through scholarships from National Counsel for Scientific and Technological Development (CNPq), my first year, and Sao Paulo Research Foundation (FAPESP) for each progressive year, made my studies possible, thank you.

To Professor Simone Alves Silva (UFRB) for introducing me to "Science" when I was undergraduate student. Thank you for all that you have done for me.

Last, but not least, I am so grateful to all of my friends for their support, encouragement, friendship, and help throughout this whole process. Special thanks needs to be given to Márcia Carvalho, Marcela Muller, Paula Caroline, Jonathas Graças, Ronaldo Sakai, Yuri Ramos, Naiara Célida, Gentil Cavalheiro, Cleucione Pessoa, Marilda (New Planet Idiomas), Ana Schwantes, Halan Vieira, Rafael Labigalini, Karla Vilaça, Diego Pequeno, Valdson Silva, Anderson Ramos, Clíssia Barboza, Carolina Andrade, Denis Costa, Rogério Lorençoni, Leonardo Cirilo, Rafael Bortolotto, Francynês Macedo, Gabriel Daneluzzi, Tiago Tezotto, Mateus Donega, Thiago Costa, Amanda Silva, Keru Chen, Hyungju Andy Park, Martin Bielich, Heather Akin, Alencar Xavier, Jason Roth, Péter Kovács, Martha Rebellon-Pavese, Maleja Bueno, Juan Rebellon, Jeff Pavese, Yolanda Montoya, Maira Aponte-Rodriguez, Cesar Garbossa, Hebert, Cristiano, Sherwood Lee, Mabel Munoz, Oscar, Eduardo Mariano, Evandro, Leonardo Cesar, and José Marcos. There are too many more people to list who I owe a great deal too, but thank you all for taking the time to help me. Words alone cannot express how appreciative I am of all of you. Thank you, for all that you have done for me.

SUMMARY

RESUMO.....	9
ABSTRACT.....	11
1 INTRODUCTION	13
1.1 Literature review.....	15
1.1.1 Importance of maize crop for Brazil.....	15
1.1.2 Breeding for environmental stress tolerance and grain yield improvement	16
1.1.3 Maize crop management aiming high yields.....	18
2.0 Principals of maize development	20
3.0 Final Consideration.....	23
References.....	23
2 MAIZE NUTRIENT UPTAKE AND PARTITIONING FOR DROUGHT AND NON- DROUGHT TOLERANT HYBRIDS INFLUENCED BY VARIED PLANT DENSITY AND NITROGEN RATE.....	31
Abstract.....	31
2.1 Introduction.....	32
2.2 Materials and Methods.....	34
2.3 Results and Discussion.....	38
2.4 Conclusions.....	62
References.....	96
3 PHYSIOLOGICAL CHARACTERIZATION OF RECENT DROUGHT-TOLERANT MAIZE HYBRIDS AT VARYING STRESS LEVELS: PHOTOSYNTHESIS, GRAIN YIELD, AND GRAIN YIELD COMPONENTS.....	105
Abstract.....	105
3.1 Introduction.....	106
3.2 Materials and Methods.....	107
3.3 Results and Discussion.....	110
3.4 Conclusions.....	117
References.....	131
4 MAIZE RESPONSE TO NITROGEN APPLICATION TIMING AND CORRELATION AMONG LEAF VARIABLES WITH GRAIN YIELD AND COMPONENTS.....	137
Abstract.....	137

4.1 Introduction.....	137
4.2 Materials and Methods.....	139
4.3 Results and Discussion.....	142
4.4 Conclusions.....	148
References.....	154
5. GENERAL DISCUSSION	157
APPENDICES.....	159

RESUMO

Impactos do nitrogênio sobre o rendimento de milho e eficiência de uso do nutriente em ambientes de estresses contrastantes

Adubação nitrogenada (N) e o estresse hídrico tem grande influência no rendimento de grãos de milho, assim, estudos sobre genótipos e o manejo dessa cultura são muito importantes para o aumento da produtividade. Este estudo é apresentado em três capítulos. Os dois primeiros foram desenvolvidos nos Estados Unidos e o terceiro no Brasil. Os objetivos são apresentados na seguinte ordem: (1) o objetivo principal foi entender quais características, se existirem, diferem entre híbridos tolerantes e não-tolerantes à seca, com semelhança em maturidade, que regem a absorção de nutrientes e concentrações destes sob diferentes tratamentos de cultivo e sua influência na produção de grãos. (2) O objetivo principal foi investigar as respostas fisiológicas (fotossíntese (*A*) e transpiração (*E*)) e a produção de milho em genótipos, com semelhante maturidade, tolerantes e não tolerantes ao déficit hídrico (P1151 vs. P1162 e P1498 vs. 33D49) em relação a variação de densidade de plantas e doses de N. (3) O principal objetivo foi investigar as respostas de milho à aplicação de nitrogênio em cobertura, ureia fertilizante (^{15}N), em diferentes estádios fenológicos. O objetivo secundário foi: verificar a correlação entre as clorofilas e carotenoides com SPAD (avaliado em V14 e V16) e destas com a biomassa total (BM), índice de colheita (IC), produção de grãos (PG) e do conteúdo de N nos grãos. Como resultados: (1 e 2) Todos os híbridos responderam de forma semelhante para PG em relação aos tratamentos. O Híbrido P1151 demonstrou semelhantes *A* e *E* e menor área foliar do que seu semelhante em maturidade (P1162). Híbrido P1498 pareceu ser capaz de manter a taxa de transpiração foliar e de fotossíntese mais elevadas do que 33D49, durante o período de enchimento de grãos. Este híbrido apresentou uma melhor persistência na captação de água pela raiz no final da estação de cultivo. Geralmente maiores BM e PG na maturidade foram relacionadas a menores intervalos de diferenciação floral e a maior acumulação, na estação seca, de macronutrientes (P e S) no colmo, sendo as últimas características consideradas como mecanismos de tolerância à seca. (3) A cultura do milho respondeu de forma semelhante em relação a PG à aplicação de N. O teor de nitrogênio nos grãos derivado do ^{15}N fertilizante e a eficiência de uso do fertilizante nitrogenado foram maiores em relação a aplicação de N nos primeiros estádios. Houve correlação positiva e significativa para SPAD com a maioria dos pigmentos no estágio de avaliação V16. Provando ser um instrumento eficaz de avaliação indireta de clorofilas e carotenóides em estágios iniciais. A clorofila b, avaliada em V16, apresentou correlação positiva significativa ($p < 0,05$) com teor de N nos grãos, PG, e BM, a clorofila total em V16 também apresentou uma correlação positiva com o teor de N nos grãos, no entanto, as clorofilas a e total, avaliadas em V14, apresentaram correlação negativa com PG. Assim, a medição do teor de pigmentos com o objetivo de estudar as condições nutricionais e prever a produção de grãos deve ser realizada após o estágio V14.

Palavras-chave: Tempo e doses de nitrogênio; Densidade de plantio; Fotossíntese; Genótipos tolerantes ao déficit hídrico

ABSTRACT

Nitrogen impacts on maize yield and nutrient use efficiency in contrasting stress environments

Nitrogen (N) fertilization and drought stress have large influence on maize grain yield, thus studies about genotypes and management technologies are very important to increase maize production. This study is presented in three chapters; the first two were undertaken in the United States of America and the third one in Brazil. The following objectives are addressed in this order: (1) The primary objective was to understand which traits, if any, differ between similar-maturity tolerant and non-drought tolerant hybrids that govern nutrient uptake and concentrations under different management treatments (varied plant densities (PD) and N rates) and their influence on grain yield (GY). (2) The primary objective was to investigate the physiological and yield responses of comparable-maturity drought and non-drought tolerant hybrids (P1151 vs. P1162, and P1498 vs. 33D49) to varied plant density and N rates. (3) The main objective was to investigate the responses of maize to sidedress N applications, using isotopically labeled urea fertilizer (^{15}N), at different development stages. The secondary objective was to verify the correlations between the chlorophylls and carotenoids with SPAD index (evaluated at V14 and V16) and all these parameters with total biomass (BM), harvest index (HI), GY and grain N content. The major results for objectives 1 and 2 were as follows: All hybrids had similar GY responses to PD (near 79,000 versus near 100,000 plants ha^{-1}) and N rate (from 0 to 269 kg N ha^{-1}) treatment factors. Hybrid 1 (AQUAmax™ P1151) demonstrated similar leaf photosynthetic (*A*) and transpiration (*E*) rates than its non-drought tolerant counterpart of similar maturity since Hybrid 2 (P1162) had a higher leaf area Index (LAI) (at the R2 and R3 stages) and a similar GY as Hybrid 1. Hybrid AQUAmax™ P1498 maintained higher leaf *A* and *E* rates than P33D49 during the grain-fill period, thus perhaps demonstrating improved persistence in root water uptake late in the season. There was no single trait differentiation in photosynthesis or transpiration between drought and non-drought tolerant hybrids. Highest BM and GY at maturity generally followed shorter anthesis-silking intervals and more stover macronutrient (P and S) accumulation, in the drought season, so these characteristics appeared to be important drought-tolerant mechanisms regardless of hybrid designations. The major findings from the objective 3 investigations: Maize crop responded similarly for GY to timing of sidedress N application. Grain N content from ^{15}N fertilizer and N uptake and efficiency were greater for early N applications. SPAD values correlated positively with most pigment variables at V16 in both seasons, thus proving that SPAD was an efficient instrument of indirect evaluation of chlorophylls and carotenoids in maize leaves at early stages. Chlorophyll b at V16, sample stage, was positively correlated ($P < 0.05$) with grain N content, GY, and BM, and total chlorophyll at V16 was positively correlated with GY and grain N content. However the chlorophylls a and total, evaluated at V14, were negatively correlated with GY. So, measurement chlorophyll and carotenoid pigment contents should be done after V14 stage when studies aim to evaluate crop nutritional conditions and prescribe future grain production practices.

Keywords: Nitrogen time and rates; Plant density; Photosynthesis; Drought tolerant genotypes

1 INTRODUCTION

Maize (*Zea mays* L.) is one of the three most cultivated grains in terms of total MT (metric ton) production in the world, with approximately 960 million tons (FAO, 2013). United States of America, China, Argentina and Brazil are the largest producers, accounting for 70% of world's production (PIONEER, 2014). To encounter the demand for food for the growing world population, a significant increase in the world grain production is required, particularly in crops grown in developing countries. Grain yield (GY) has increased during the last decades in many parts of the world as a result of agronomics practices and genetic improvement of the maize crop.

The improvement in maize GY through the last decades, mostly due to increased tolerance to crowding intensity, has indirectly been accompanied by a decline in grain N concentration (%Ng) (DUVICK, 1997; CIAMPITTI; VYN, 2012). Therefore, gains over time in N use efficiency (NUE) (yield versus applied N ratio) (MOLL et al., 1982). The average U.S. maize yield has risen at a rate of 118 kg ha⁻¹ yr⁻¹ from 1930 to 2000, GY increased from 1.5 Mg ha⁻¹ in the 1930ies to 9.5 Mg ha⁻¹ for the 2006-2008 period, and similar rates of improvement have been observed in other parts of the world (TOLLENAAR; LEE, 2011).

Furthermore, greater GY of newer maize hybrids might have resulted in a concomitant increase in resource capture and/or resource use efficiency. In the same way, GY increases can be explained as a result of higher tolerance of hybrids to different categories of environmental stresses associated to the efficient use of nutrients and higher plant densities. The water limited availability (drought) is the main stress factor limiting crop production (SEGHATOLESLAMI KAFI.; MAJIDI, 2008; GOLBASHY et al., 2010). Drought is a permanent constraint to agricultural production in many developing countries, and an occasional cause of losses of agricultural production in developed countries (GOLBASHY et al., 2010).

In some years, yield can be significantly reduced by transient water limitations of varying timing, duration, and severity. Many of these water limitations have minor to moderate impact on yield. However, widespread and continued drought that substantially reduces grain yield over a wide area can occur in some years (BOYER et al., 2013). When maize encounters water deficits, there is a decline in the photosynthesis per plant. This can be due to a reduction in light interception as leaf expansion is reduced or as leaves senesce, and to reductions in C fixation per unit leaf area as stomata close or as photo-oxidation damages the photosynthetic mechanism (LEUNG; GIRAUDAT, 1998; MUGO; BÄNZIGER.;

EDMEADES, 2000). Therefore, one of the main goals in breeding programs is selection of the best genotypes under drought stress conditions (RICHARDS et al., 2002; MORADI et al., 2012). Consequently, seed companies in response to these problems apply diverse strategies to improve crop tolerance to drought stress (COOPER et al., 2014).

Past research has studied the physiological effects of water stress in maize (SANCHEZ et al., 1983; ÇAKIR, 2004; MARKELZ; OSTERMAN; MITCHELL, 2011) as well as physiological traits that may confer increased drought tolerance (BÄZINGER et al., 2000; CAMPOS et al., 2006; LOPES et al., 2011). Therefore, little public and physiology-focused research publications are available that have investigated these recently released drought-tolerant hybrids.

Grain yield is a complex trait and is dependent on many factors including vigorous growth, adequate water and nutrient supplies, enhanced solar radiation interception and conversion to chemical energy, and improved genetics (RUSSELL, 1991). Nutrient, mainly Nitrogen (N), use efficiency is one important factor for maize production, because the N stress reduces GY by delaying plant growth and development (UHART; ANDRADE, 1995) and reducing the leaf area index, leaf area duration, and photosynthetic rate (SINCLAIR; HORIE, 1989; CONNOR et al., 1993) among other negative factors.

Management techniques and breeding approaches for N use efficiency differ in different production conditions and regions in the world. Accordingly, the understanding of the process that governs crop N uptake and its distribution in plants is of main importance to optimize crop production with minimum N input (CASSMAN et al., 2002). Studies about macro- and micro-nutrient uptake and partitioning, such N, P, K and S and Zn, Fe, Mn and Cu for modern maize hybrids are also important to understand the mechanisms that govern nutrient assimilation and their influence on grain yield under different environmental conditions.

For N management studies it is also important to give attention to the evaluation methods of this nutrient in the plant. Traditional methods used to determine the amount of chlorophyll in the leaf require destruction of tissue samples and much work in the extraction and quantification processes. The development of a portable chlorophyll meter, which allows instantaneous measurements of the N amount corresponding to its content in the leaf without destroying it, is an alternative to estimate the relative content of these pigments in the leaf (DWYER et al., 1991; ARGENTA et al, 2001). The knowledge about the effective influence of the factors that determine the performance of the plant can contribute decisively to minimize the stress caused by nitrogen deficiency. Thus, it is extremely important to obtain

more reviews related to the correlation between the real content of chlorophyll and real carotenoids with the values obtained by indirect chlorophyll (SPAD) measurements in the early development stages of the maize.

This study is presented in three chapters. The first two were developed in the United States of America and the third one in Brazil. The following objectives are presented in this order: (1) the primary objective was to understand which traits, if any, differ between similar maturity-tolerant and non-drought tolerant hybrids that govern the nutrient uptake and concentrations under different management treatments (varied plant densities and N rates) and their influence on GY. The second objective was to evaluate if the drought-tolerant hybrids have an overall higher N recovery and use efficiencies, produce more grain per unit of whole-plant nutrient uptake, and achieve higher N, P, K and S internal efficiency and indexes, compared to the non-drought tolerant hybrids at varied plant densities and N rates. (2) The primary objective was to investigate the physiological and yield responses of comparable-maturity drought and non-drought tolerant hybrids (P1151 vs. P1162, and P1498 vs. 33D49) to varied plant density and N rates. The secondary objective was to specifically examine leaf photosynthesis (*A*) and transpiration (*E*) rates of hybrids across the growing season in response to varied PD and N rate treatments. (3) The main objective was to investigate the responses of maize to the nitrogen application, urea fertilizer (^{15}N), in side-dress at different development stages. The secondary objective was: verifying the correlation between the chlorophylls and carotenoids with the SPAD index and these ones with total biomass, harvest index (HI), grain yield (GY) and grain N content in response to the nitrogen side-dress in different development stages.

1.1 Literature review

1.1.1 Importance of maize crop for Brazil

Maize production in Brazil shows a growth rate of about 4% per year. Cultivated under different cropping systems, maize is grown mainly in the Center-West, Southeast and South regions, in the states of Paraná, Mato Grosso, Minas Gerais, Goiás, Mato Grosso do Sul, and Rio Grande do Sul, Brazil. A study of the production projection of this cereal made by “Assessoria de Gestão Estratégica do Mapa”, indicates an increase of 19.11 million tons between harvests of 2008/2009 and 2019/2020. In 2019/2020, a production of 70.12 million tons is expected, and for 2022/2023, 93.6 million tons (BRASIL, 2014).

The productive chain of maize is one of the most important of the Brazilian agribusiness, corresponding to 37% of the national grain production. The growing demand for grain, both nationally and internationally, reinforces the high potential of the sector. Together with soybean, maize is the basic input for poultry and swine feed, two very competitive markets that generate revenues to Brazil (BRASIL, 2007). There is an ever-growing demand for maize due to the recent and fast development of Asiatic countries, increasing grain consumption for animal feed and for alcohol production in the United States.

1.1.2 Breeding for environmental stresses tolerance and grain yield improvement

In the seed market, the maize genotypes availability with high yields and tolerance to abiotic stresses characteristics is a challenge for the breeding programs. Plants adaptation to adverse environments or situations under suboptimal environmental factors involves an adaptation to multiple stresses that comes together with direct and indirect interactions. Thus it is very important to identify and characterize genotypes, as well as studies on the interaction and overlapping mechanisms, both physiologically as biochemical and molecular (DURÃES et al., 2004).

Satisfactory results have been obtained with maize, improving the drought tolerance in genotypes (MONNEVEUX et al., 2006), efforts in the architecture of plants has also been made for improvements in the root system, to improve water absorption and nutrients and disposal of leaves, in order to improve sunlight interception of over the canopy.

In relation to plant physiology, the improvements can be attributed to several factors such as maintenance and longevity leaves state "stay-green" (THOMAS; HOWARTH, 2000). The progress of leaf senescence can normally be observed by naked eye when there is chlorophyll loss, so that the expression "stay-green" is generically assigned to an individual when the senescence shows up later in relation to a reference genotype (THOMAS; SMART, 1993). Besides being related to water stress tolerance, the "stay green" state increases the tolerance to insects and diseases, makes the crop more tolerant to a higher plant density, being consequently related to productivity.

The efficiency in the uptake, partitioning and effective use of nutrients by the plant is also very important for the production of maize. The efficiency in fertilizer use depends on the ability of the roots of the plant has to obtain high concentrations of nutrients that are available in the soil. Thus, in order to obtain more effective root systems, breeders have studied variations in root system morphology and kinetic parameters of maize genotypes

(BALIGAR; BARBER, 1979; ANGHINONI et al., 1989), highlighting the importance of handling of them in breeding programs to obtain more efficient cultivars in nutrient absorption. The root system morphology is determined by the length, volume, area and radius of the roots and root hairs (SCHENK; BARBER, 1979) that varies in direct proportion to the length and thickness of roots because these attributes influence the absorption surface (VILELA; ANGHINONI, 1984).

Other factors that have provided improvements in grain maize production are the high activity of the source, leaves, and ability to meet the demand of the drains, ears (TOLLENAAR; WU, 1999), in which the leaves have shown greater photosynthetic capacity, and retaining capacity of nitrogen uptake by the plant with extended nutrient accumulation during the reproductive stage (CIAMPITTI; VYN, 2012), the older hybrids the highest rate of nitrogen absorption was observed in periods prior the flowering period.

1.1.3 Maize crop management aiming high yields

1.1.3.1 Tillage influence in maize production

Several agronomic practices are employed to increase grain production in maize, among them is important the soil preparation aiming weed control to favor crop development. However, the intensive use of the soil can lead to the formation of compacted soil layers, to the reduction of soil aggregate stability, and to the formation of a larger number of microspores what may induce to a greater tendency for soil loss by erosion. In this way, the maintenance of crop residues at soil surface in the minimum tillage management system provides a better water retention and a better protection against the direct impact of rain drops (IGUE, 1984), in comparison to the residue incorporation made in the conventional soil preparation.

Minimum tillage has been highlighted among the most employed management technologies for maize cultivation, mainly due to the awareness of farmers of the need for a better quality of soils, looking to a more sustainable agriculture (COELHO, 2006). This type of management is characterized by crop seeding without revolving the soil, crop rotation and residue maintenance on the soil surface (PEREIRA et al., 2009). Minimum tillage studies have observed a greater efficiency in relation to conventional soil preparation because the avoiding of soil revolving leads to a less intense and gradual decomposition of crop residues (CARVALHO et al., 2004), therefore improving chemical, physical and biological soil characteristics with positive effects on soil fertility, reducing inputs of lime and fertilizer.

1.1.3.2 Nitrogen and other nutrients: influence in maize grain production

Nutrients have different translocation rates in stem, leaf and grain. In relation to nutrient export, phosphorus (P) is highly transferred to grain (77 to 86 %), followed by N (70 to 77 %), sulfur (S) (about 60 %), magnesium (Mg) (47 to 69 %), potassium (K) (26 to 43 %) and calcium (Ca) (3 to 7 %). Anyway, the incorporation of plant residues returns a great part of the absorbed nutrients, mainly K and Ca from the straw (COELHO, 2006). Maize crop removes large quantities of nutrients from the soil, according to a review (STEWART et al., 2013) which documents that the increase of 57% of the grain production from 1960 to 2000 can be attributed to fertilizer imputes into the production system. It is usual to employ N fertilization as a soil cover application to complement the N supplied by the soil when high productivities are aimed (CANTARELLA, 1993).

The recommended levels of NPK in Brazil for productivities above 8 t ha⁻¹ is of 10 to 20 kg ha⁻¹ of N at planting time, 120 kg ha⁻¹, 100 kg ha⁻¹, or 70 kg ha⁻¹ of P₂O₅ for low, medium and high availabilities of P, respectively. For K, recommended rates are 90 kg ha⁻¹, 80 kg ha⁻¹ and 60 kg ha⁻¹ of K₂O, for low, medium and high availabilities, respectively (RIBEIRO et al., 1999). Important practices are related to N application timing and planting date. Several authors support that the best moment for N application is at seeding time in one single dose. However, the majority of the soil fertility scholars say that the N application has to be split in two doses, one at seeding and the other after emergence, during the vegetative period of the plant.

The nitrogen requirements for maize are considerably variable and different in each plant development stage (ARNON, 1975). Although, it is known that the crop requires about 20 kg N ha⁻¹ for each ton of grain produced (FANCELLI, 2000; SOUSA; LOBATO, 2004) there are still many controversies and discussions regarding the ideal time of application, and about the maximum ideal N rate for this crop. Cantarella (1993) states that even if the N absorption by maize is intense 40 to 60 days after emergence, the plant still absorbs about 50% of the N that is needed after the beginning of flowering. The author comments that there might be advantages in a late application of the N in cases of heavy fertilization, very sandy soils or irrigated areas.

However, studying N application timing for corn, Neptune and Campanelli (1980), observed that the higher grain yield was obtained when all of the N was applied at the planting, and the production decreased when the N was applied from 73 to 83 days after the emergency. Even though, Coelho (1987) reported that the full implementation of N rates at the planting caused higher level of corn dry matter per kg of N than the gain obtained from the N applied as sidedressing. França et al. (1994) reported that the splittings of N did not affect the efficiency of nitrogen fertilizer, and the results obtained were similar when 106 kg of N per ha were applied in a single dose, at the stage where the plant has 6 leaves (V6 stage), or subdivided in half applied in the 6 leaves stage and the other half in the stage where the plant has 10 leaves (V10 stage). These authors also concluded that most of the N in the plant is accumulated until anthesis, reaching values of up to 93%, they concluded that the nitrogen sidedressing should be made after seeding and before early anthesis, during which the rate of absorption is virtually linear.

1.1.3.3 Plant density influence on maize grain yield

Planting density is one of the most important factors determining grain productivity of the maize crop. This crop is the most sensitive member of the family Poaceas in relation to plant density (ALMEIDA; SANGOI, 1996). The increase of the population density is one way of maximizing solar radiation interception. The ideal plant density depends of the chosen cultivar, soil fertility, water availability and sowing time. Therefore, productivity tends to increase in relation to plant population, until a certain number of plants per area, considered as the optimum population. Above this number the productivity decreases as the number of plants per ha increases (PEREIRA, 1991). Among the forms of manipulation of the spatial arrangement, plant density is having the greatest interference in maize yields, because small changes in the population can affect significantly grain yield per hectare. This response occurs because the maize does not have a space compensation mechanism as efficient as other (ANDRADE et al., 1999).

Therefore, high plant densities can reduce the photosynthetic activity of the crop and, consequently, the efficiency of the conversion of the photosyntates to grain production; can increase the interval between anthesis and silk, and can reduce the number of grains per ears (SANGOI et al., 2003). Nonetheless, plant densities below the optimum lead to a lower use of the incident solar radiation decreasing productivity. Management taking into account plant density lead to an increase in maize productivity due to a better use of environmental factors

by the modern genotypes found in the market (DOURADO NETO et al., 2003). The increase of plant density up to a given limit is a technique used to increase maize grain yield. The ideal number of plants per ha is variable, once the maize plant alters grain yield according to the interspecific competition resulting from different plant densities (PEIXOTO et al., 1996).

Recent reports indicate that the frequently used optimum plant density aimed for maize grain yield is in the United States of America approximately 8 plants m^{-2} (80,000 plants ha^{-1}) (TOLLENAR; LEE, 2011), and in Brazil only 3.5 to 5 plants m^{-2} , corresponding to 55,000 to 72,000 plants ha^{-1} . Therefore, maize breeding programs have been sought for genotypes with high response to production at high population densities from 80,000 to 100,000 plants per hectare, and under lower spacing between lines (DOURADO NETO et al., 2003).

2.0 Principles of maize development

During the vegetative period which extends from seeding to the beginning of the appearance of reproductive organs, the maize plant accumulates dry matter through photosynthesis. For this process the plant needs CO_2 from the atmosphere, absorbed through stomata. Therefore leaves are an essential part of the plant, the following factors being of extreme importance for plant productivity: number of open stomata per unit leaf area; leaf area (represented by the “leaf area index” - LAI); number of leaves per plant; plant density (PD), indicating the population of plants per ha; plant architecture; plant height (PH); and concentration of chlorophyll and carotenoid pigments, among others. These factors are directly related to solar energy capture for photosynthesis (photo-chemical reaction).

Plants also need H_2O for photosynthesis, which comes from the soil so that the root system becomes important. Its length, surface area, and depth into the soil are very important factors. The root system is also important for nutrient absorption, such as: nitrogen, phosphorus, potassium, sulfur, calcium, and all other essential nutrients. The sugars fixed by photosynthesis are responsible for the dry matter accumulation (Plant biomass - BM) and finally for grain yield (GY). During the vegetative phase, these carbohydrates are allocated initially to roots, then to stalks and leaves, as shown schematically in the Figure 1.1.

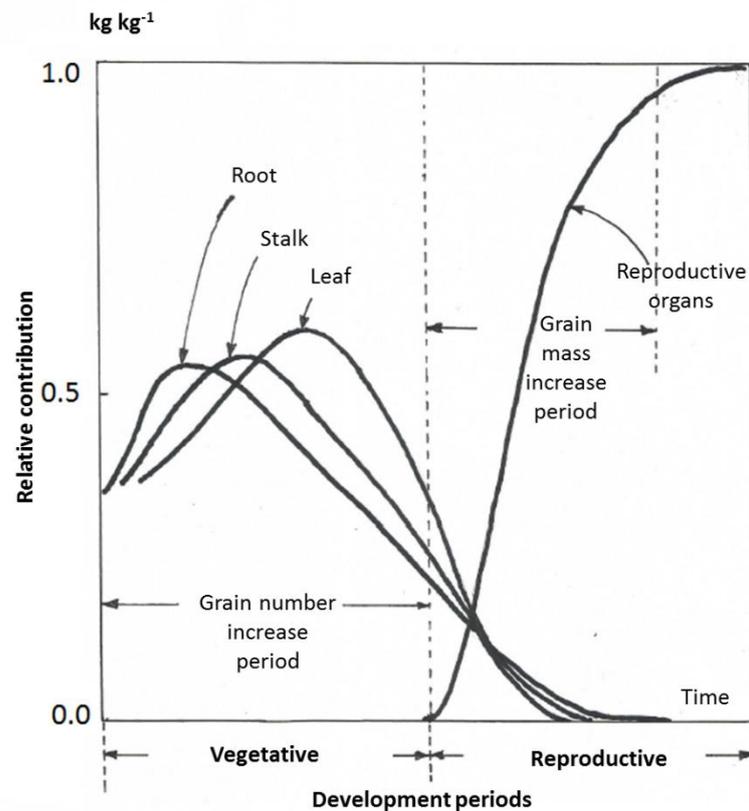


Figure 1.1 - Plant Biomass and grain yield influenced by the sugar translocation and nutrient partitioning from different plant organs.

The dry matter accumulation, and consequently of all nutrients and its partitioning, during the vegetative period, are of extreme importance for plant “health” and are crucial processes for the final yield determination. In the case of maize, the kernel number (KN) is determined during this phase, and logically, the larger the better GY. When the reproductive organs start to be developed, the CO₂ partitioning almost stops for roots, stalks, and leaves. From that moment this CO₂ flow turns to the reproductive organs, as shown schematically above. During the reproductive period the already defined kernel number in the vegetative period, will gain in weight or kernel weight (KW) (grain filling), which is important for the final GY.

When all soil-plant-atmosphere conditions are in the optimum range, the crop tends its maximum potential yield at a given location. Deviations from the optimum are the result of stresses. These can be of different causes. Water deficit conditions, when the actual evapotranspiration surpasses rainfall, induce hydric stress. Nutrient stresses occur when the plant cannot obtain the needed amount of a given nutrient. In the case of nitrogen, for

example, splitting of the fertilizer dose are chosen to avoid N stress at given moments of crop development. Competition among plants for nutrients or water is also an important type of stress. Therefore, the stand of a crop, defined by the number of plants per ha, called plant density (PD), is very important in crop management. The soil-plant-atmosphere local conditions define the ideal values of PD. Just comparing the State of São Paulo, Brazil, with the State of Indiana, USA, in average terms, we can say that in the first soils are poor. They present CEC values of the order of 50 (meq/100g soil), with low surface organic matter, low water availability (100 to 120 mm per m of soil profile), with possible daylight hours from 11 to 13, so that no high PDs can be adopted (of the order of 50 to 70,000 plants per ha). Therefore our commercial Brazilian yields seldom reach 12,000 kg ha⁻¹ of maize grain. On the other hand, soils of Indiana are richer as well as in organic matter as in fertility (CEC of the order of 300 (meq/100g soil)), and with a much higher water availability (200 to 300 mm per m of soil profile) and 12 to 14 daylight hours. They can sustain PDs of 120,000 plants per ha, or more, achieving commercial productivities over 20,000 kg ha⁻¹. Figure 1.2 discusses schematically effects of PD on yield, in the presence of other stresses.

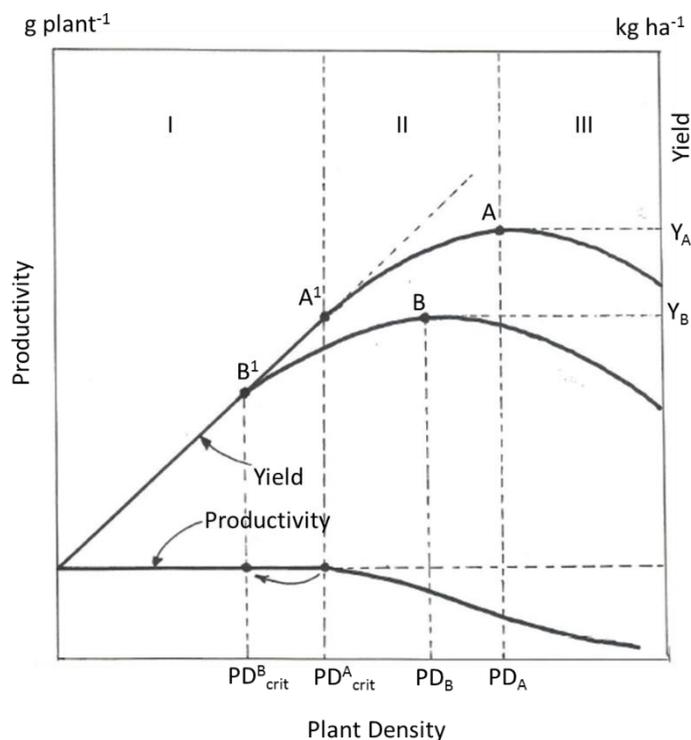


Figure 1.2 - Schema - Plant density (PD) and different environmental stress influences on maize grain yield

According to this figure, under a given soil-plant-atmosphere condition, the plant productivity remains constant up to the point when PD reaches a critical value (PD_{crit}^A at A)

and they start competing among themselves for water, nutrients, etc, reaching a maximum yield Y_A with PD_A . For a lower PD_B (at point B) the yield is decreased to Y_B and a lower PD_{crit}^B is established.

These are, in very short terms, the principles that govern maize development and productivity. Since yield is our final goal, the study of the factors affecting it is of extreme importance, mainly for newly developed hybrids. This study, comprised into three chapters, presents there experiments that test different maize hybrids, mainly in relation to effects on grain yield caused by drought and nitrogen stresses (Table 1.1).

Table 1.1 - Research characterization, treatment factors

Country	Hybrid	Drouth-tolerance	Plant population* (pl ha ⁻¹)	Highest GY productivity (t ha ⁻¹)*
Brazil	30F35HR	No	60,000	11.10
USA	AQUAmax ^T	Yes	78,000	13.3
	^M P1151 HR			
USA	P1162 HR	No	99,000	13.6
USA	AQUAmax ^T	Yes	78,000	12.7
	^M P1498 HR			
USA	33D49 HR	No	78,000	11.7

* GY of the best treatment of one of the three experiments

3.0 Final Consideration

Brazilian production and productivity of the maize crop suffered very significant increases in Brazil, following a worldwide trend, which begun in the last century. This is the result of studies in various fields of research with responses in production technology of the crop and in the use of modern genotypes. There is, however, still need of more research aiming for a better understanding of the productive and physiologic mechanisms of the new hybrids appearing in the market in response to contrasting environments, in order to ensure a more sustainable production respecting natural resources.

References

ALMEIDA, M.L. de; SANGOI, L. Aumento da densidade de plantas de milho para regiões de curta estação estival de crescimento. **Pesquisa Agropecuária Gaúcha**, Porto Alegre, v. 2, n. 2, p. 179-183, 1996.

AMADO, T.J.C.; MIELNICZUK, J.; AITA, C. Recomendação de adubação nitrogenada para o milho no RS e SC adaptada ao uso de culturas de cobertura do solo, sob sistema plantio direto. **Revista Brasileira de Ciência do Solo**, Viçosa, v. 26, p. 241-248, 2002.

ANDRADE, F.H.; VEGA, C.; UHART, S.O. Kernel number determination in maize. **Crop Science**, Madison, v. 39, p. 453-459, 1999.

ANGHINONI, I.; VOLKART, K.; FATTORE, C.; ERNANI, P.R. Morfologia de raízes e cinética da absorção de nutrientes em diversas espécies e genótipos de plantas. **Revista Brasileira de Ciência do Solo**, Viçosa, v. 13, p. 355-361, 1989.

ARNON, I. **Mineral nutrition of maize**. Bern: International Potash Institute, 1975. 452 p.

BALIGAR, V.C.; BARBER, S.A. Genotypic differences of corn for ion uptake. **Agronomy Journal**, Madison, v. 71, p. 870-873, 1979.

BÄNZINGER, M.; EDMEADES, G.O.; BECK, D.; BELLON, M. **Breeding for drought and nitrogen stress tolerance in maize: from theory to practice**. Mexico: CIMMYT, 2000. 68 p.

BERNARDI, A.C.C.; MACHADO, P.L.O.A.; FREITAS, P.L.; COELHO, M.R.; LEANDRO, W.M.; OLIVEIRA JÚNIOR, J.P.; OLIVEIRA, R.P.; SANTOS, H.G.; MADARI, B.E.; CARVALHO, M.C.S. **Correção do solo e adubação no sistema de plantio direto nos cerrados**. Rio de Janeiro: Embrapa Solos, 2003. 22 p. (Documentos, 46).

BOYER, J.S.; BYRNE, P.; CASSMAN, K.G.; COOPER, M.; DELMER, D.; GREENE, T.; GRUIS, F.; HABBEN, J.; HAUSMANN, N.; KENNY, N.; LAFITTE, R.; PASZKIEWICZ, S.; PORTER, D.; SCHLEGEL, A.; SCHUSSLER, J.; SETTER, T.; SHANAHAN, J.; SHARP, R.E.; VYN, T.J.; WARNER, D.; GAFFNEY, J. The US drought of 2012 in perspective: a call to action. **Global Food Security**. West Chester, v. 2, p. 139-143. 2013.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. **Cadeia produtiva do milho**. Brasília: IICA; MAPA, SPA, 2007. 140 p.

_____. Disponível em: <<http://www.agricultura.gov.br/vegetal/culturas/milho>>. Acesso em: 06 ago. 2014.

ÇAKIR, R. Effect of water stress at different development stages on vegetative and reproductive growth of corn. **Field Crops Research**, Aberdeenshire, v. 89, n. 1, p. 1-16, 2004.

CAMPOS, H.; COOPER, M.; EDMEADES, G.O.; LOFFLER, C.; SCHUSSLER, J.R.; IBANEZ, M. Changes in drought tolerance in maize associated with fifty years of breeding for yield in the U.S. Corn Belt. **Maydica**, Bergamo, v. 51, p. 369-381, 2006.

CANTARELLA, H. Calagem e adubação do milho. In: BÜLL, L.T.; CANTARELLA, H. (Ed.). **Cultura do milho: fatores que afetam a produtividade**. Piracicaba: POTAFOS, 1993. p. 148-196.

CARVALHO, M.A.C. de; SORATTO, R.P.; ATHAYDE, M.L.F.; SÁ, M.E. Produtividade do milho em sucessão a adubos verdes no sistema de plantio direto e convencional. **Pesquisa Agropecuária Brasileira**, Brasília, v. 39, p. 47-53, 2004.

CASSMAN, K.G.; DOBERMANN, A.; WALTERS, D.T.; YANG, H. Meeting cereal demand while protecting natural resources and improving environmental quality. **Annual Review of Environment and Resources**, Palo Alto, v. 28, p. 315-358, 2003.

CIAMPITTI, I.A.; VYN, T.J. Physiological perspectives of changes over time in maize yield dependency on nitrogen uptake and associated nitrogen efficiencies: a review. **Field Crops Research**, Aberdeenshire, v. 133, p. 48–67, 2012.

CIAMPITTI, I.A.; ZHANG, H.; FRIEDEMANN, P; VYN, T.J. Potential physiological frameworks for mid-season field phenotyping of final plant nitrogen uptake, nitrogen use efficiency, and grain yield in maize. **Crop Science**, Madison, v. 52, p. 2728-2742, 2012.

COELHO, A.M. **Balço de nitrogênio (15 N) na cultura do milho (*Zea mays* L.) em um Latossolo Vermelho Escuro fase cerrado**. 1987. 142 p. Dissertação (Mestrado em Solos e Nutrição de Plantas) – Escola Superior de Agricultura de Lavras, Lavras, 1987.

_____. **Nutrição e adubação do milho**. Sete Lagoas: Embrapa CNPMS, 2006. 10 p. (Circular Técnica, 78).

COELHO, A.M.; CRUZ, J.C.; PEREIRA FILHO, I.A. Rendimento do milho no Brasil: chegamos ao máximo? **Informações Agrônomicas**, Piracicaba, n. 101, p. 1-12, mar. 2003. Encarte Técnico.

COMPANHIA NACIONAL DE ABASTECIMENTO. **Acompanhamento da safra brasileira: grãos**. Oitavo levantamento – junho de 2012. Brasília, 2012. 34p.

CONNOR, D.J., HALL, A.J.; V.O. SADRAS. Effect of nitrogen content on the photosynthetic characteristics of sunflower leaves. **Australian Journal of Plant Physiology**, Oxford, v. 20, p. 251–263, 1993.

COOPER, M.; GHO, C.; LEAFGREN, R.; TANG, T.; MESSINA, C. Breeding drought-tolerant maize hybrids for the US corn-belt: discovery to product. **Journal of Experimental Botany**, Oxford, v. 65, n. 21, p. 6191-204, 2014.

DOURADO NETO, D.; FANCELLI, A.L. **Produção de milho**. Guaíba: Agropecuária, 2000. 360 p.

DOURADO NETO, D.; PALHARES, M.; VIEIRA, P.A.; MANFRON, P.A.; MEDEIROS, S.L.P.; ROMANO, M.R. Efeito da população de plantas e do espaçamento sobre a produtividade de milho. **Revista Brasileira de Milho e Sorgo**, Sete Lagoas, v. 2, n. 3, p. 63-77, 2003.

DURÃES, F.O.M.; SANTOS, M.X.; GAMA, E.E.G.; MAGALHÃES, P.C.; ALBUQUERQUE, P.E.P.; GUIMARÃES, C.T. **Fenotipagem associada a tolerância a seca em milho para uso em melhoramento, estudos genômicos e seleção assistida por**

marcadores. Sete Lagoas: Embrapa Milho e Sorgo, 2004. 17 p. (Embrapa Milho e Sorgo. Circular Técnica, 39).

DUVICK, D.N. What is yield? In: EDMEADES, G.O.; BANZIGER, M.; MICKELSON, H.R.; PENA-VALDIVIA, C.B. (Ed.). **Developing drought- and low N-tolerant maize.** Mexico: CIMMYT, 1996. p. 332–335.

DWYER, L.M.; TOLLENAAR, M.; HOUWING, L. A nondestructive method to monitor leaf greenness in corn. **Canadian Journal of Plant Science**, Ottawa, v. 71, p. 505-509, 1991.

FANCELLI, A.L. **Nutrição e adubação do milho.** Piracicaba: ESALQ, 2000. 43 p.

FAOSTAT. Disponível em: <<http://faostat.fao.org/>>. Acesso em: 01 dez. 2013.

FNP CONSULTORIA E COMÉRCIO. Balanço 2008 & perspectivas 2009. In: _____. **AGRIANUAL 2009:** anuário da agricultura brasileira. 14. ed. São Paulo, 2009. p. 371-376.

FRANÇA, G.E.; COELHO, A.M.; RESENDE, M.; BAHIA FILHO, A.F.C. Parcelamento da adubação nitrogenada em cobertura na cultura do milho irrigado. In: EMBRAPA. Centro Nacional de Pesquisa de Milho e Sorgo. **Relatório técnico anual do Centro Nacional de Pesquisa de Milho e Sorgo:** 1992-1993. Sete Lagoas: 1994. p. 28-29.

GOLBASHY, M.; EBRAHIMI, M.; KHAVARI-KHORASANI, S.; CHOUCAN, R. Evaluation of drought tolerance of some corn (*Zea mays* L.) hybrids in Iran. **African Journal of Agricultural Research**, Nairobi, v. 5, n. 19, p. 2714-2719, 2010.

IGUE, K. **Dinâmica da matéria orgânica e seus efeitos nas propriedades do solo.** In: ADUBAÇÃO verde no Brasil. Campinas: Fundação Cargill, 1984. p. 232-267.

LEUNG, J; GIRAUDAT, J. Abscisic acid signal transduction. **Annual Review of Plant Physiology and Plant Molecular Biology**, Palo Alto, v. 49, p. 199–222, 1998.

LOPES, M.S.; ARAUS, J.L.; PHILIPPUS, D.R.VH., FOYER, C.H. Enhancing drought tolerance in C4 crops. **Journal of Experimental Botany**, Oxford, v. 62, p. 3135-3153. 2011.

MARKELZ, R.J.C.; STRELLNER, R.S.; LEAKEY A.D.B. Impairment of C4 photosynthesis by drought is exacerbated by limiting nitrogen and ameliorated by elevated [CO₂] in maize. **Journal of Experimental Botany**, Oxford, v. 62, p. 3235-3246, 2011.

MARKWELL, J.; OSTERMAN, J.C.; MITCHELL, J.L. Calibration of the Minolta SPAD-502 leaf chlorophyll meter. **Photosynthesis Research**, Dordrecht, v. 46, p. 467-472, 1995.

MOLL, R.H.; KAMPRATH; E.J.; JACKSON, W.A. Analysis and interpretation of factors which contribute to efficiency of nitrogen utilization. **Agronomy Journal**, Madison, v. 74, p. 562–564, 1982.

MONNEVEUX, P.; SANCHEZ, C.; BECK, D.; EDMEADES, G. O. Drought tolerance improvement in tropical maize source populations: evidence of progress. **Crop Science**, Madison, v. 46, p. 180-191, 2006.

MORADI, H.; AKBARI, G.A.; KHAVARI, K.S.; RAMSHINI, H.A. Investigation of drought stress effect on morphologic traits, yield and yield components of corn (*Zea Mays* L.) new hybrids. **International Journal of Recent Scientific Research**, Sorappur, v. 3, n. 6, p. 518-529, 2012.

MUGO, S.N.; BÄNZIGER, M.; EDMEADES, G.O. Prospects of using ABA in selection for drought tolerance in cereal crops. In: WORKSHOP ON MOLECULAR APPROACHES FOR THE GENETIC IMPROVEMENT OF CEREALS FOR STABLE PRODUCTION IN WATER-LIMITED ENVIRONMENTS: A STRATEGIC PLANNING, 1999, El Batan. **Proceedings...** Mexico: CIMMYT, 2000. p. 73–78. 2000.

NEPTUNE, A.M.L.; CAMPANELLI, A. Efeitos de épocas e modo de aplicação do sulfato de amônio - 15 N, fósforo - 32 P, na quantidade e teores de N, P e K na planta e na folha do milho, na produção, na quantidade de proteína e eficiência do nitrogênio do fertilizante convertido em proteína. **Anais da Escola Superior de Agricultura Luiz de Queiroz**, Piracicaba, v. 37, n. 2, p. 1105-1143, 1980.

PEIXOTO, C.M. **Resposta de genótipos de milho à densidade de plantas, em dois níveis de manejo**. 1996. 118 p. Dissertação (Mestrado em Agronomia) - Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, 1996.

PEREIRA, R.G.; ALBUQUERQUE, A.W.; MADALENA, J.A.S. Influência dos sistemas de manejo do solo sobre os componentes de produção do milho e *Brachiaria decumbens*. **Revista Caatinga**, Mossoró v. 22, p. 64-71, 2009.

PEREIRA, R.S.B. Caracteres correlacionados com a produção e suas alterações no melhoramento genético do milho (*Zea mays* L.). **Pesquisa Agropecuária Brasileira**, Brasília, v. 26, p. 745-751, 1991.

PIONEER. Disponível em: <<http://www.pioneersementes.com.br/MediaCenter/Pages/Detalhe-do-Artigo.aspx?p=165>>. Acesso em: 08 jul. 2014.

RIBEIRO, A.C.; GUIMARÃES, P.T.G.; ALVAREZ V., V.H. **Recomendação para o uso de corretivos e fertilizantes em Minas Gerais: 5ª aproximação**. Viçosa: Comissão de Fertilidade do Solo do Estado de Minas Gerais, 1999. 359 p.

RICHARDS, R.A.; REBETZKE, G.J.; CONDON, A.G.; VAN HERWAARDEN, A.F. Breeding opportunities for increasing the efficiency of water use and crop yield in temperate cereals. **Crop Science**, Madison, v. 42, p. 111-121, 2002.

ROTH, J.A.; CIAMPITTI, I.A.; VYN, T.J. Physiological evaluations of recent drought tolerant maize hybrids at varying stress levels. **Agronomy Journal**, Madison, v. 5, n. 4, p. 1129 – 1141, 2013.

RUSSELL, W.A. Genetic improvements of maize yields. **Advances in Agronomy**, San Diego, v. 46, p. 245–298, 1991.

SÁ, J.C.M. **Manejo de nitrogênio na cultura do milho no sistema plantio direto**. Passo Fundo: Aldeia Norte, 1996. 23 p.

SANCHEZ, R.A.; HALL, A.J.; TRAPANI, N.; HUNAU, R. C. de. Effects of water stress on chlorophyll content, nitrogen level and photosynthesis of leaves of two maize genotypes. **Photosynthesis Research**, Dordrecht, v. 4, p. 35-47, 1983.

SANGOI, L.; SILVA, P.R.F.; ARGENTA, G.; HORN, D. Bases morfo-fisiológicas para aumentar a tolerância de cultivares de milho a altas densidades de plantas. In: REUNIÃO TÉCNICA CATARINENSE DE MILHO E FEIJÃO, 4., 2003, Lages. **Resumos expandidos...** Lages: CAV, UDESC, 2003. p. 19-24.

SCHENK, M.K.; BARBER, S.A. Phosphate uptake by corn as affected by soil characteristics and root morphology. **Soil Science Society of America Journal**, Madison, v. 43, p. 880-883, 1979.

SEGHATOLESLAMI, M.J.; KAFI, M.; MAJIDI, S. Effect of drought stress at different growth stage on yield and water use efficiency of five proso millet (*Panicum Miliaceum* L.) genotypes. **Pakistan Journal of Botany**, Karachi, v. 40, n. 4, p. 1427- 1432, 2008.

SINCLAIR, T.R.; HORIE, T. Leaf nitrogen, photosynthesis, and crop radiation use efficiency: a review. **Crop Science**, Madison, v. 29, p. 90-98, 1989.

SOUSA, D.M.G. de; LOBATO, E. Calagem e adubação para culturas anuais e semiperenes. In: _____. (Ed.). **Cerrado: correção do solo e adubação**. Planaltina: Embrapa Cerrados, 2004. p. 283-315.

SOUZA, C.M. **Efeito do uso contínuo de grade pesada sobre algumas características físicas e químicas de um Latossolo Vermelho-Amarelo Distrófico, fase cerrado, e sobre o desenvolvimento das plantas e absorção de nutrientes pela cultura de soja**. 1988. 105 p. Dissertação (Mestrado em Fitotecnia) - Universidade Federal de Viçosa, Viçosa, 1988.

STEWART, W.M.; DIBB, D.W.; JOHNSTON, A.E.; SMYTH, T.J. The contribution of commercial fertilizer nutrients to food production. **Agronomy Journal**, Madison, v. 97, p. 1-6, 2005.

THOMAS, H.; HOWARTH, C.J. Five ways to stay green. **Journal of Experimental Botany**, Oxford, v. 51, p. 329-337. 2000.

THOMAS, H; SMART C.M. Crops that stay green. **Annals of Applied Biology**, Oxford, v. 123, p. 193-219, 1993.

TOLLENAAR, M.; LEE, E.A. Strategies for enhancing grain yield in maize. **Plant Breeding Reviews**, Hoboken, v. 34, p. 37-81, 2011.

TOLLENAAR, M.; WU, J. Yield improvement in temperate maize is attributable to greater stress tolerance. **Crop Science**, Madison, v. 39, p. 1597-1604, 1999.

UHART, S.A.; ANDRADE, F.H. Nitrogen deficiency in maize: I. Effects on crop growth, development, dry matter partitioning, and kernel set. **Crop Science**, Madison, v. 35, p. 1376-1383, 1995.

2 MAIZE NUTRIENT UPTAKE AND PARTITIONING FOR DROUGHT AND NON-DROUGHT TOLERANT HYBRIDS INFLUENCED BY VARIED PLANT DENSITY AND NITROGEN RATE

Abstract

Improvements in maize (*Zea mays* L.) drought tolerance are vital for maintaining local and global food security. The research objectives were to understand whether and, if so, how similar maturity-tolerant and non-drought tolerant hybrids differ in nutrient uptake, nutrient concentrations and grain yield (GY) under different management treatments (varied plant density and N rates) in rain-fed environments. Our specific focus was to evaluate if drought-tolerant hybrids have overall higher N recovery and N use efficiencies, produce more grain per unit of whole plant nutrient uptake, and achieve higher macro-nutrient efficiencies, compared to non-drought tolerant hybrids. Two drought-tolerant (AQUAmax) hybrids were compared to two comparable-maturity hybrids (non-drought tolerant) at varying plant density (PD) (two levels) and N rates (four levels) over 2 years (2012 and 2013) in Northwestern Indiana. Morphological, productivity and nutritional measurements were taken in both seasons. All hybrids, whether labeled as more drought tolerant or less so, responded similarly in grain yield to the treatments factors in both years. AQUAmax hybrids did not demonstrate improvement in grain yield or more yield stability than non-AQUAmax. The drought-tolerant Hybrid 1 (P1151) generally displayed a longer anthesis to silking interval than comparable-maturity Hybrid P1162 (in 2013). In 2012, both drought-tolerant hybrids presented higher values for stover P content than the non-drought tolerant. As the same way, drought-tolerant Hybrid P1498, in the significant factorial for Hybrid x PD, presented higher stover S content than the Hybrid 33D49, this specific quality found in drought-tolerant hybrids could be considered as a drought-tolerance mechanism presented in this AQUAmax hybrid. Hybrid P1498 generally showed a slightly earlier flowering, and a lower anthesis-to-silking interval, than hybrid 33D49 in both seasons. Hybrid 1498 also had consistently lower kernel numbers but higher final kernel weights than 33D49 (both years). In 2013, the majority of the macronutrient harvest indices were higher than those observed in 2012. The PHI was lower than normal in 2012, but also significantly lower in drought-tolerant hybrids than in the two more drought susceptible hybrids. There was no evidence that AQUAmax hybrids were any different than non-AQUAmax hybrids in their GY, BM, and yield stability responses to N fertilizers or in their macronutrient use efficiencies. Agronomic-optimum N fertilizer rates are not lower for these AQUAmax hybrids. However, because hybrid, N, and PD treatment combinations with highest BM and GY at maturity generally achieved shorter anthesis-silking intervals and, in the drought season, more stover macronutrient accumulation (P and S), the latter traits appeared to be important drought-tolerant mechanisms regardless of hybrid designations. There was no evidence that AQUAmax hybrids were any different than non-AQUAmax hybrids in their response to N fertilizers or in their N use efficiencies. Thus it is unlikely that N fertilizer management should change when AQUAmax hybrids are grown. Certainly there is no evidence that optimum N fertilizer rates would be any lower for these AQUAmax hybrids.

Keywords: Nitrogen use efficiency; Drought tolerance mechanism; Grain yield

2.1 Introduction

Commercial maize (*Zea mays* L.) yields have increased substantially as a result of conventional breeding and agronomic practices. The average U.S. maize yield has risen at a rate of 118 kg ha⁻¹ yr⁻¹ from 1930 to 2000, GY increased from 1.5 Mg ha⁻¹ in the 1930s to 9.5 Mg ha⁻¹ for the 2006-2008 period, and similar rates of improvement in maize GY have been observed in other parts of the world (TOLLENAAR; LEE, 2011).

Grain yield increases have also been attributed to greater stress tolerance of modern hybrids, especially stress from interplant competition (TOKATLIDIS; KOUTROUBAS, 2004). Plant density is an agronomic factor that changed significantly during the past decades, suggesting that the selection under high plant density was the key to improve maize GY (TROYER; ROSENBROOK, 1983). The tolerance to plant density is in essence the tolerance to the resource scarcity of water, mineral nutrients and light (YAN; WALLACE, 1995).

Despite the high yield potential, maize has a marked sensitivity to biotic and abiotic stresses; therefore, its cultivation needs careful planning and management, aiming to maximize its productive capacity (Andrade, 1995). Two important environmental stresses that decrease corn grain yield (GY) are (a) drought, specifically during the critical period two weeks prior post-silking, and (b) nitrogen (N) deficiency, as N stress reduces crop photosynthesis (BÄNZIGER et al., 2000) and also influences the uptake and internal efficiency of other macronutrients at maturity (CIAMPITTI et al., 2013a).

Rising greenhouse gas concentrations increases the global temperatures that could potentially accelerate maize growth and development, hasten maturity and reduce soil moisture availability during the growing season. Therefore, improvements in maize drought tolerance are vital for maintaining local and global food security (BOOMSMA; VYN, 2008). Additionally improving maize nitrogen use efficiency would especially benefit many developing countries, where yields are low and fertilizer application inadequate (AKINTOYE; KLING; LUCAS, 1999; CASSMAN et al., 2004).

Maize productivity relies on carbon and nitrogen (N) metabolic activity. The direct role of N in the accumulation of grain dry matter is represented by affecting maize dry matter production, influencing the development and maintenance of the crop leaf area and photosynthetic efficiency (BELOW et al, 1981; SWANK et al, 1982; MUCHOW, 1998). This nutrient has important functions in maize plant metabolism, participating as part of protein molecules, coenzymes, nucleic acids, cytochromes, and chlorophyll, among others. Nitrogen

fertilization influences not only maize productivity, but also the quality of the product as a result of increased grain protein content (SABATA; MASON, 1992).

When subjected to drought, corn plants exhibit a lower water potential in relation to non-stressed plants, causing stomatal closure and, consequently, decreased photosynthesis and carbon assimilation (OTEGUI; ANDRADE; SUERO., 1995). The main effects of water shortages on maize plants are decreased dry matter (biomass) production and grain yield (KORSAKOV et al., 2008).

Seed companies, in response to these problems, are applying diverse strategies to improve crop tolerance to drought stress (COOPER et al., 2014). In regions where the drought stress is present, drought-tolerant hybrids have demonstrated yield benefits when compared with the non-drought-tolerant hybrids with similar maturity (BECKER et al. 2012; PIONEER, 2013). Management techniques and breeding approaches for N use efficiency differ in different production conditions and regions in the world. Accordingly, the understanding of the process that governs crop N uptake and its distribution in plants is of main importance to optimize crop production with minimum N input (CASSMAN; DOBERMANN; WALTERS, 2002). Studies about macro- and micro-nutrient uptake and partitioning, such N, P, K and S and Zn, Fe, Mn and Cu for modern maize hybrids are also important to understand the mechanisms that govern nutrient assimilation and their influence on grain yield under different environmental conditions.

The primary objective of this study was to understand which traits, if any, differ between similar maturity-tolerant and non-drought tolerant hybrids that govern the nutrient uptake and concentrations under different management treatments (varied plant density and N rates) and its influence on GY. The second objective was to evaluate if the drought-tolerant hybrids have an overall higher N recovery and use efficiencies, produce more grain per unit of whole-plant nutrient uptake, and achieve higher N, P, K and S internal efficiency and indexes, compared to the non-drought tolerant hybrids at varied plant densities and N rates.

The following questions highlight the research objectives that examined the effects of genotypes, plant densities and N rates on the underlying physiology of maize response to PD and N stress factors: (1) Are the drought-tolerant hybrids different from their comparative-maturity counterparts without the drought tolerance? (2) Do the drought-tolerant hybrids respond differently to N rates when it comes not only just to yield but especially when it comes to nutrient uptake, and does the drought-tolerant characteristic mean that there is an increased capacity for nutrient uptake? (3) If we can expect an improvement in water use efficiency, can we then also expect that the plant will be able to accumulate more nutrients

even at a lower overall N rate; if this occurs, would we see a situation develop where the drought-tolerant hybrids lead to improved nutrient use efficiency as well as better water use efficiency? (4) How do the drought tolerant hybrids differ from their similar-maturity hybrids in their nutrient uptake response to N rates and response to plant density? (5) How are these hybrids different in their allocation of nutrients, the harvest index, and internal efficiencies, compared to the non-drought tolerant hybrids?

2.2 Materials and Methods

2.2.1 Location and Experimental Design

The experiment was conducted over two growing seasons (2012-13) in northwest Indiana at the Pinney Purdue Agricultural Center (PPAC) (41° 26' 49" N, 86° 55' 42" W). Non-irrigated field-experiments were established on the Tracy sandy loam (coarse-loamy, mixed mesic Ultic Hapludalfs). In both seasons, the preceding crop was soybean [*Glycine max* (L.) Merr.], and the tillage employed was fall chisel plow and spring secondary tillage. The 2012 season experiment was planted on May 11th, and the 2013 season experiment was planted on May 1st.

A five replicate split-split plot design was utilized with hybrid as main plot, plant density (PD) as the subplot, and N rate as the sub-subplots. In both seasons, four hybrids were compared consisting of two pairings with different drought-tolerance: 111 CRM hybrids (AQUAmaxTM P1151 HR (Hybrid 1) versus P1162 HR (Hybrid 2), and 114 CRM hybrids (AQUAmaxTM P1498 HR (Hybrid 3) versus 33D49 HR (Hybrid 4). Drought tolerance scores, as determined by DuPont Pioneer on a nine point scale (1 = low, 9 = high), for P1151 and P1498 were both 9, and for the hybrids less drought-tolerant, P1162 and 33D49, were 8 and 7, respectively. The two PD levels were 79,000 (PD1) and 104,000 (PD2) pl ha⁻¹ for 2012, and 78,000 (PD1) and 99,000 (PD2) plants ha⁻¹ final stand for 2013.

All plots received 26 kg N ha⁻¹ in a 5-cm x 5cm starter band (19-17-0) at planting. Side-dress Urea Ammonium Nitrate, UAN, (28-0-0) treatments of either 0 (Nr1), 134 (Nr2), 202 (Nr3), or 269 (Nr4) kg N ha⁻¹ were coulter-injected between corn rows around the V5 growth stage (ABENDROTH et al., 2011) in both years. Intensive measurements were performed in three replicates whose individual plots measured 4.6 meters wide (six 76.2 cm rows) by 27 meters long (18 m long for the remaining two replicates). Soil was sampled from the 0 to 30cm layer, collecting 20 cores (2-cm diameter) from non-fertilized plots, for soil mineral N concentration determination, before and after UAN side-dress application. Soil samples (0 to 20 cm depth) from each replication for general fertility data were analyzed by

A&L Great Lakes Laboratories (Table 2.1); the resultant mean data indicates rather similar soil organic matter and soil-test P concentrations between the 2 site-years, and that soil-test K concentrations were above the critical levels in both years. Data about weather conditions were recorded in both seasons (Figure 2.1).

Table 2.1 - Soil analysis for non-fertilized plots (inorganic nitrogen [NO_3^- - N / NH_4^+ - N], organic matter content [OM], soil pH, potassium content [K], and phosphorus, Bray – P1 [P]) in the uppermost 0.3 m of the soil profile for each growing season, 2012 (Roth et al., 2013) and 2013. At PPAC, Wanatah, IN, United States

Soil parameters	Growing seasons	
	2012	2013
OM content, g kg^{-1}	16 (3.0)	17.05 (0.33)
pH units	6.10	6.59
P, mg P kg^{-1}	43 (9.4)	48.45 (11.13)
K, mg K kg^{-1}	103 (13.8)	134.60 (25.19)

The value within parenthesis refers to standard deviation

2.2.2 Morpho-Physiological Plant Measurements

Twenty continuous plants at representative plant densities were selected and marked from the center rows of each plot (sampling areas) for repeated measurements during each growing season. Plant heights were measured from the soil surface to the highest vertically-extended leaf during the vegetative stages. At flowering (R1) plant heights were recorded up to the uppermost developed leaf collar (MADDONNI; OTEGUI, 2004; BOOMSMA, 2009). In 2012 height measurements were performed at the V5, V10, V15, and R1 stages, while in 2013 measurements were performed at the V5, V10 and V12 stages. Stalk diameter measurements were determined at various vegetative and reproductive stages (V10, V15, R1, R3, R4 – 2012; V10, V12, V14 – 2013) by using a Mitutoyo ABSOLUTE Digimatic 500-171 caliper (Mitutoyo America Corporation, Aurora, IL) connected to a Personal Digital Assistant (PDA) and recorded into a spreadsheet. Calipers were placed at the widest inter-nodal midpoint on plant stems between the lowest above-ground nodes without brace roots. A Minolta SPAD-502 Chlorophyll Meter (Minolta Sensing Americas, Inc., Ramsey, NJ) was used for estimating chlorophyll content of plants (SPAD). One measurement was taken from the most fully expanded (collared) leaf during vegetative stages (~ V10 and V15) and from the ear leaf at reproductive stages (R1, R3, R4, and R5) in the 2012 growth season, and (V10, V12 and V14) in the 2013 growth season. For both years measurements were performed on all 20 plants and the average value per plot was recorded. If the designated leaf was damaged,

the next lower leaf was used for measurement. Measurements were taken near the middle of each leaf, about 2 cm from the leaf edge, and areas of non-representative leaf-necrosis were avoided.

Information gathered on the 20 plants at flowering included anther suspension and silk extrusion. Plots were monitored each day at the beginning of the tassel period (prior to silk extrusion for hybrids evaluated) and observations continued until all plants completed silking. Plants were counted as being at anthesis when at least 10 anthers were suspended from the tassel, and at silking when silks extruded at least 1 cm from the husk. A 10-90% anthesis (or silking) duration was calculated by subtracting the dates at which 2 plants (10%) had reached anthesis (or silking) from the date at which at least 18 plants (90%) had reached anthesis (or silking). The anthesis-silking interval (ASI) was calculated by subtracting the date at which 10 plants (50%) reached anthesis from the date at which 10 plants (50%) reached silking.

2.2.3 Grain Yield and Yield Components

A methodology identical to the one used by Roth, Ciampitti, and Vyn. (2013) was followed. At maturity, GY and its components were determined in both seasons. Whole-plant biomass (BM, procedure described by Burzaco, Ciampitti, and Vyn (2014)) and Harvest Index (HI) were calculated from 10 plants harvested from the center rows of each treatment from three replications. Actual kernel number (KN) per plant and kernel weight (KW) (adjusted to 155 g kg⁻¹) were determined from ears of the consecutive 20 plants ears from the center rows at R6 three replications in each treatment. GY (also adjusted to 155 g kg⁻¹) were measured after harvesting the central two rows of each plot with a Kincaid XP plot combine.

2.2.4 Plant Nutrient Measurements

Plant nutrient content measurements were determined from the sampling areas as described by Burzaco, Ciampitti, and Vyn (2014) and Ciampitti and Vyn (2012). Plant N uptake was determined by sampling the total plant aboveground biomass (plant biomass) at the physiological maturation stage (R6), from three of five replicates in each growth season. The nutrient content was evaluated using 10 plants for stover (stem + leaves) and 20 plants for grain and cob, from three replications for stover and grain (but only from the first replication for cob). Grain, cob and stover were weighed from each plot after shelling and drying. Unfortunately, due to funding restrictions, cob fractions were only ground and submitted for nutrient analysis from the first replication. For replicates 2 and 3, cob nutrient concentrations were estimated from replicate 1 data and then adjusted for each individual plot cob weight.

These plants were cut at the stem base, weighed, and dried to achieve a constant weight at 60°C. Total N determination was performed via combustion method (AOAC International, 2000, Method 990.03).

Nutrient quantities (g plant⁻¹) of N, P, K, S, Zn, Fe, Mn and Cu in the grain, cob, and stover components were calculated by multiplying the nutrient concentration (g g⁻¹) by the dry weight (g plant⁻¹) of each fraction. Nutrient harvest indices were calculated for N (NHI), P (PHI), K (KHI), S (SHI), Zn (ZnHI), Fe (FeHI), Mn (MnHI) and Cu (CuHI) by dividing the grain nutrient content by the total plant nutrient content. The internal efficiencies of N, P, K and S (NIE, PIE, KIE and SIE, respectively), were calculated following a procedure outlined by Ciampitti and Vyn (2012) in which GY was divided by the total plant nutrient uptake in the above-ground components. In this study only N, P, K and S data were discussed but all data about nutrients are presented in the Appendix. To calculate the nitrogen use efficiency (NUE) eq. 1, and nitrogen recovery efficiency (NRE) eq. 2, the procedure by Burzaco et al. (2014) was followed:

$$NUE = \frac{GYN - GY0}{\Delta N_{applied}} \quad (1)$$

$$NRE = \frac{PNUN - PNU0}{\Delta N_{applied}} \quad (2)$$

Where GYN is the GY of N-fertilized plots, GY0 is the GY of unfertilized plots, PNUN is the PNU (total plant nitrogen uptake) of N-fertilized plots, PNU0 is the PNU of unfertilized plots, and $\Delta N_{applied}$ is the differential of N applied (N rates).

2.2.5 Statistical Analyses

Analysis of variance (ANOVA) was performed using the SAS PROC MIXED (SAS Institute, 2004) program to evaluate if significant differences existed for treatment means. The analysis was based on the factors of Hybrid, PD and N rate. All these factors were considered fixed factors (with blocks as a random factor). For model evaluation, differences between linear functions were tested (F test, Mead et al. 1993). For the total plant biomass (BM) relationships to total plant nutrient uptake were determined via regression analyses to see the possible influence of individual nutrients on total plant biomass.

2.3 Results and discussion

2.3.1 Phenology and Growing Seasons

The time for phenological development varied between the two growing seasons due to different planting dates and climatic conditions (Figure 2.1). The 2012 season experienced above-normal temperatures and record drought conditions (with only 61 mm of rain from June 1 to mid-July), which resulted in severe plant stress that was most evident during the V12-R1 interval, while the 2013 season experienced near normal temperatures and precipitation (relative to historical trends referred to by Ciampitti and Vyn (2011)) with 304 mm of rainfall occurring during the critical months of June and July. The timing of anthesis and silk emergence (Figure 2.2) was slightly different between the seasons; in 2012 plant flowering (anther suspension and silk extrusion) happened early for all hybrids in relation to 2013. In 2013 (the normal precipitation year) there was a shorter period between anthesis and silk emergence in comparison with the 2012 season (drought year). Hybrid 4 was the last hybrid to shed pollen and silk in both years.

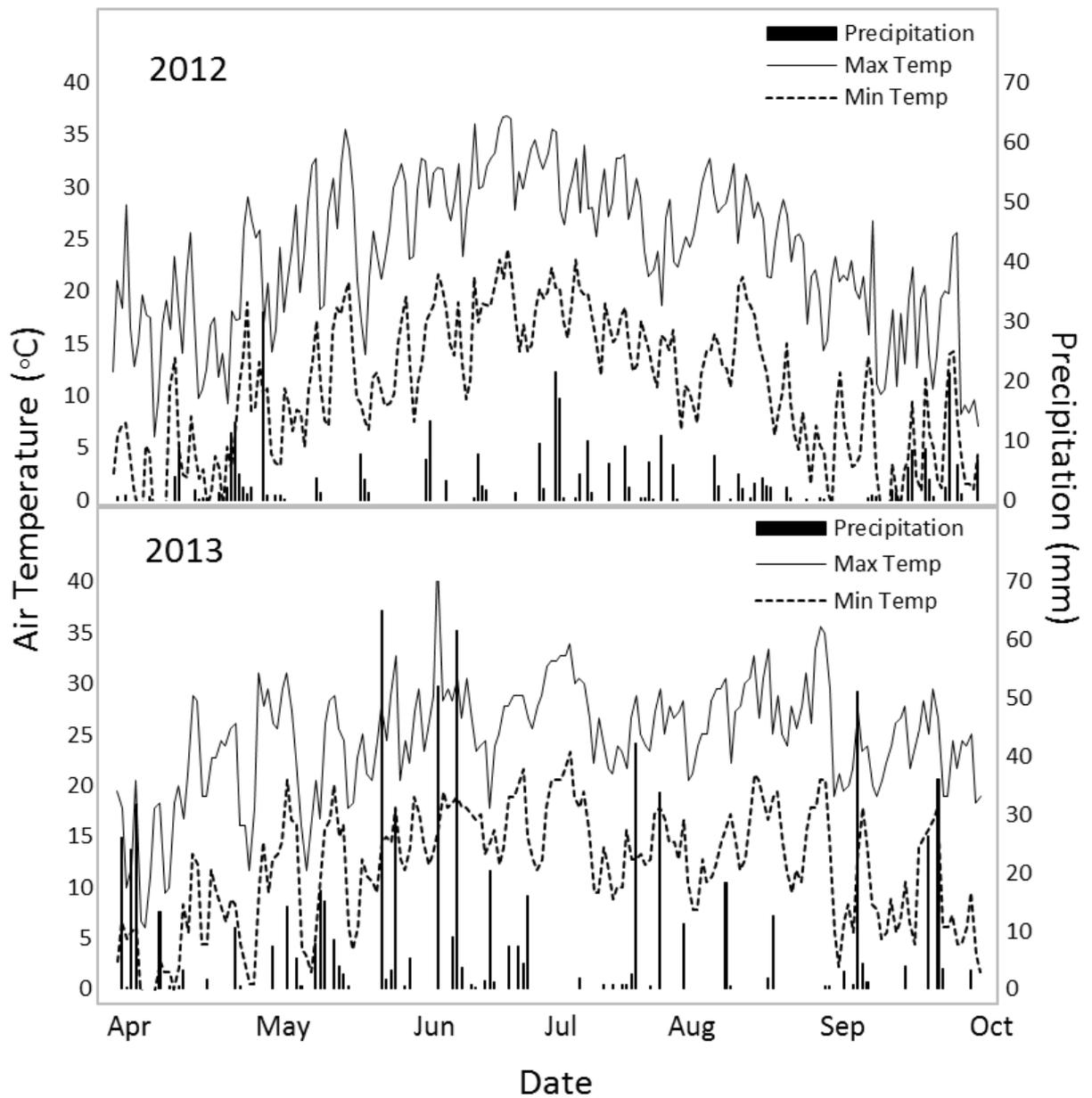


Figure 2.1 - Weather conditions (maximum and minimum air temperature and mean precipitation) for 2012 and 2013 maize growing seasons at Pinney-Purdue Agricultural Center (PPAC) in northwestern Indiana, USA

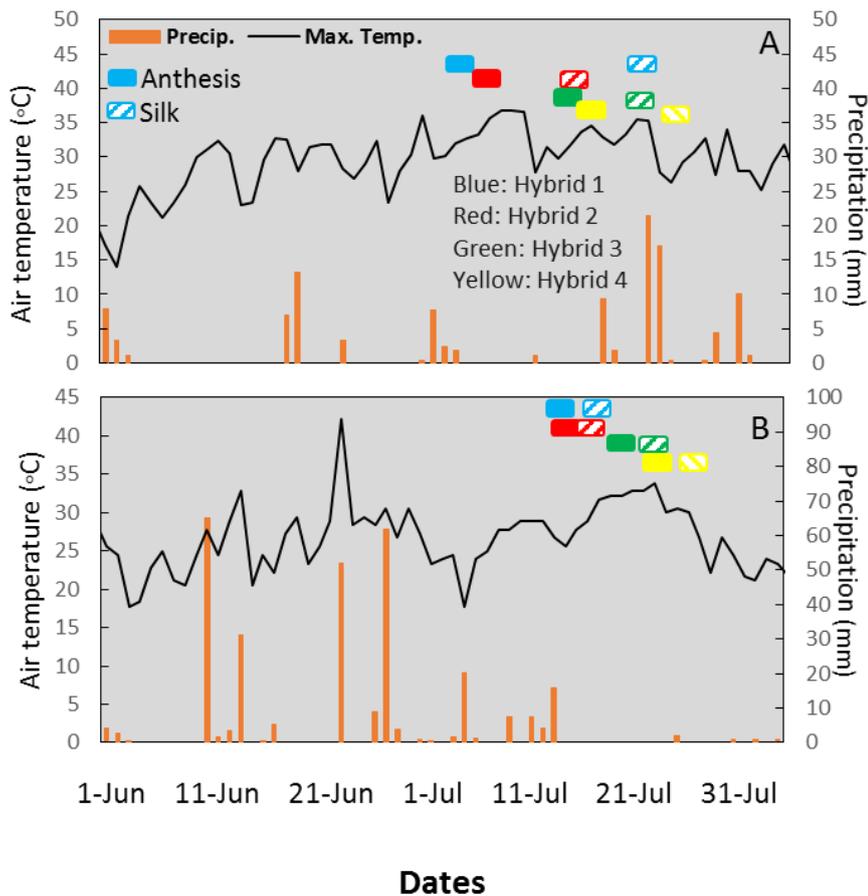


Figure 2.2 - Timing of anthesis and silk emergence (both show the day of 50%), for A (2012) and B (2013) seasons in four hybrids (Hybrid 1 = AQUAmax™ P1151, Hybrid 2 = P1162, Hybrid 3 = AQUAmax™ P1498, Hybrid 4 = 33D49) averaged across each plant density level (PD1 = 79,000, and PD2 = 104,000 pl ha⁻¹, for 2012 season; and PD1 = 78,000, and PD2 = 99,000 pl ha⁻¹, for 2013 season), and all four N rate levels (Nr1 = 0, Nr2 = 134, Nr3 = 202, and Nr4 = 269 kg N sidedress ha⁻¹) as related to maximum temperature and precipitation before and after the flowering period

2.3.2 Morpho-Physiological Plant Measurements

2.3.2.1 Plant Height and Stalk Diameter

In 2012, only slight stem elongation occurred between V10 and V15 growth stages (in a time interval of 14 days) due to drought conditions that occurred during this period (Figure 2.3; Appendix Table 5.1). At the V15 and R1 stages, PD1 demonstrated higher average heights than PD2, possibly because the higher plant density resulted in more plant growth stress under the prevailing drought conditions. A significant interaction between hybrid and PD was observed at V10, V15, and R1 stages (Appendix Table 5.3). Hybrid 2 seemed to be most negatively influenced by the PD, at R1 the Hybrid 2 plant height value was 20 cm less in PD2 compared in PD1, while the height for Hybrid 1 was numerically greater in PD2. This

ability of Hybrid 1 to conserve the higher plant height in higher PD compared with the inability of Hybrid 2, could reflect a strategy of water conservation. It is very interesting that both drought-susceptible hybrids (2 and 4) had significantly lower mean plant heights at the V15 and R1 stages for PD2 than for PD1, while there was no significant drop in plant height at the higher plant density in their drought-tolerant counterparts (Hybrids 1 and 3, respectively).

In the 2013 season, plant heights were significantly influenced by the hybrids (all sampled stages), and N rates, at V10 and V15 (Figure 2.4; Appendix Table 5.2). At V5 the Hybrid 1 was shorter than Hybrid 2, however there was no difference between Hybrids 3 and 4. There was no evidence in 2013 of height differences between drought-tolerant and drought-susceptible hybrids at later growth stages (V10 and V15). The zero N rate averaged significantly shorter plant heights at all sampling stages, and there were no hybrid interactions with either PD or N rate in 2013.

The “normal response” for maize at higher PD is for increased plant heights (SANGOI; SALVADOR, 1998; SANGOI; SILVA; ARGENTA, 2002) due to competition for light (APHALO et al., 1999), but in our population ranges we observed either a negative influence of increased PD (for the drought-susceptible hybrids in 2012) or a neutral influence of PD on plant heights (2013) .

The stalk diameter in 2012 was mainly affected by hybrid and PD, and was very little affected by N rates (Figure 2.5; Appendix Table 5.1). Hybrid 1 had consistently wider stalks than others (especially relative to Hybrids 2 and 3) from V15 to R4. As anticipated, higher PD negatively affected the stalk diameter at all growth stages measured. In 2013 (Figure 2.6; Appendix Table 5.2), all treatment factors significantly influenced the stalk diameters. Hybrid 3 had a smaller stalk diameter in relation to the other hybrids at the V10 and V14 stages. In a similar way to the 2012 season, in 2013 the stalks were wider diameter in PD1 than in PD2. The N rate factor significantly impacted the stalk diameter at all sampled stages in 2013, and especially so at V14, when the stalk value was greatest for the N rate 4. In 2012, a significant interaction between hybrid versus PD, at R1 and R4 stages, was observed (Appendix Table 5.3) because much smaller values were observed for Hybrid 2 when the plant density changed from PD1 to PD2. While in 2013, a hybrid by PD interaction was observed only at the V10 stage (Appendix Table 5.4), because Hybrids 3 and 4 had smaller stalk diameter than Hybrids 1 and 2 at PD1, whereas no hybrid differences in stalk diameter were observed at PD2.

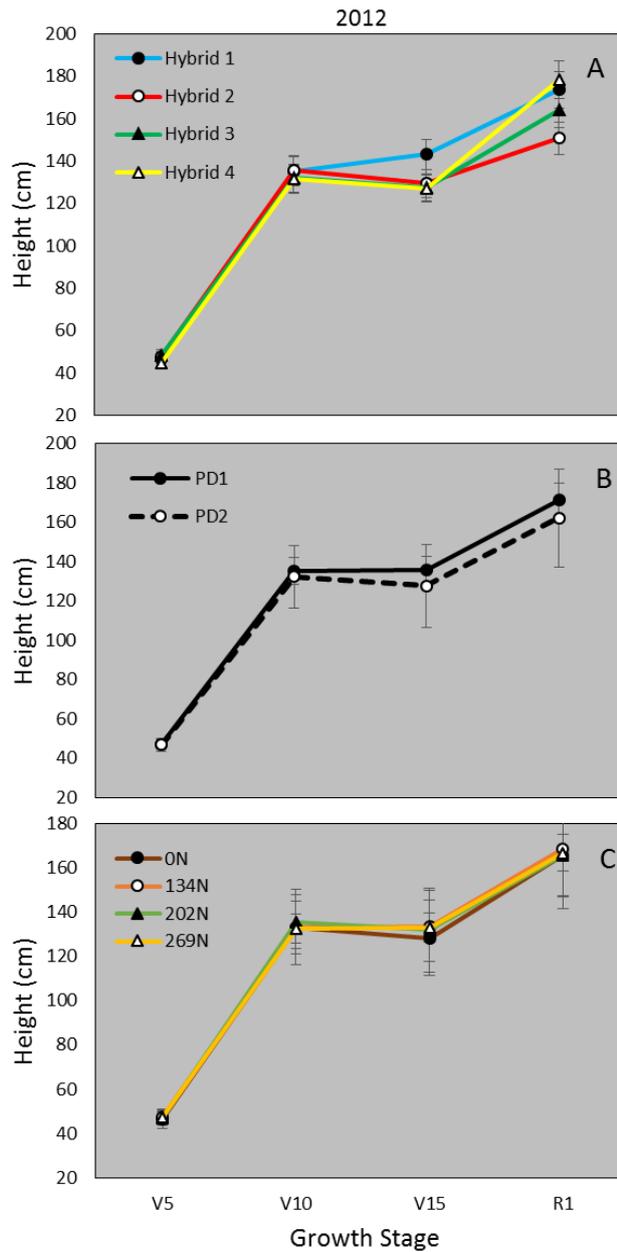


Figure 2.3 - Mean treatment effects on plant height (cm) for all four hybrids (Hybrid 1 = AQUAmax™ P1151, Hybrid 2 = P1162, Hybrid 3 = AQUAmax™ P1498, Hybrid 4 = 33D49), each plant density level (PD1 = 79,000; and PD2 = 104,000 pl ha⁻¹), and all four N rate levels (Nr1 = 0, Nr2 = 134, Nr3 = 202, and Nr4 = 269 kg N sidedress ha⁻¹) at four growth stages (V5, V10, V15, and R1) at the PPAC experimental site, for the 2012 growing season . Panel A = hybrid effect (averaged over PDs and N rates), Panel B = PD effect (averaged over hybrids and N rates), Panel C = N rate effect (averaged over hybrids and PDs)

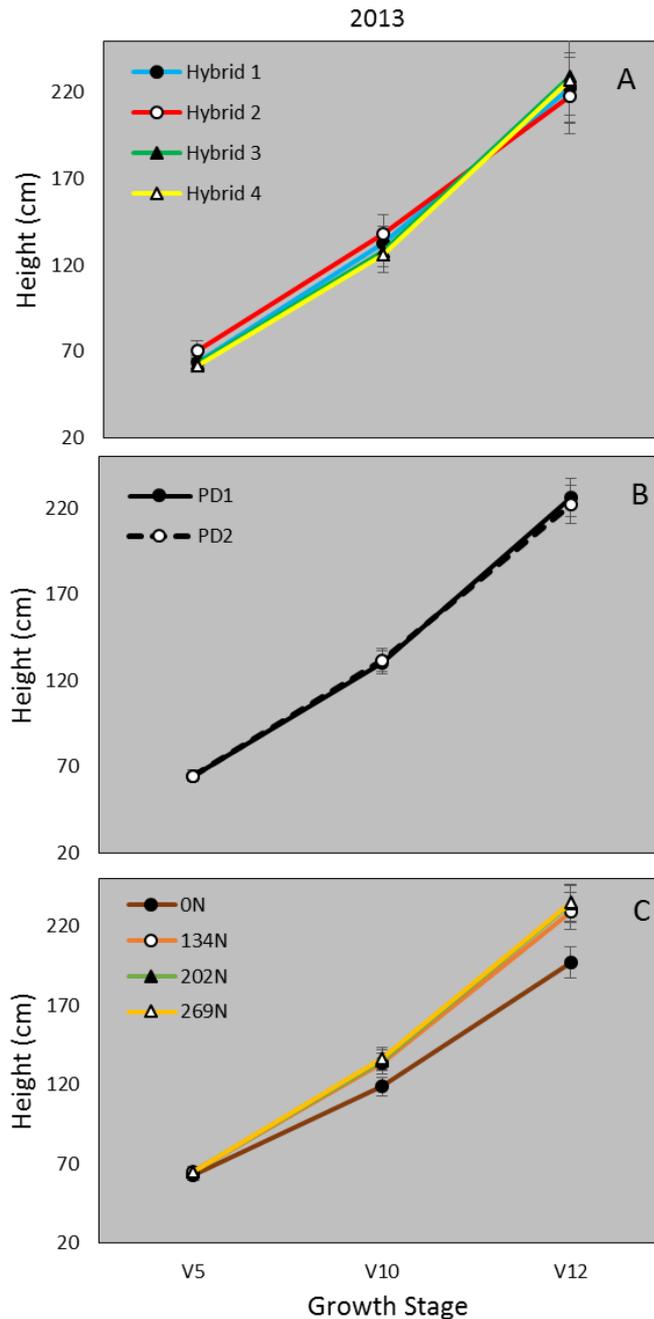


Figure 2.4 - Mean treatment effects on plant height (cm) for all four hybrids (Hybrid 1 = AQUAmax™ P1151, Hybrid 2 = P1162, Hybrid 3 = AQUAmax™ P1498, Hybrid 4 = 33D49), each plant density level (PD1 = 78,000; and PD2 = 99,000 pl ha⁻¹), and all four N rate levels (Nr1 = 0, Nr2 = 134, Nr3 = 202, and Nr4 = 269 kg N sidedress ha⁻¹) at four growth stages (V5, V10, V15, and R1) at the PPAC experimental site, for the 2013 growing season. Panel A = hybrid effect (averaged over PDs and N rates), Panel B = PD effect (averaged over hybrids and N rates), Panel C = N rate effect (averaged over hybrids and PDs)

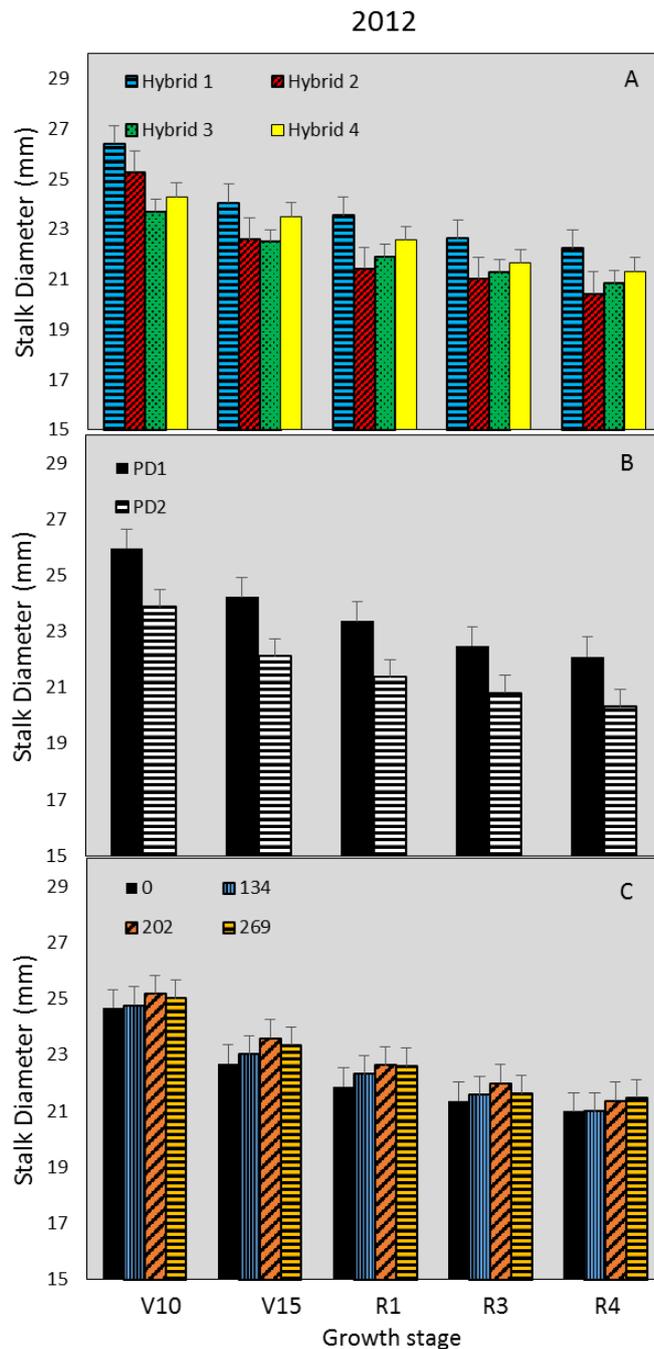


Figure 2.5 - Mean treatment effects on stalk diameter (mm) for all four hybrids (Hybrid 1 = AQUAmax™ P1151, Hybrid 2 = P1162, Hybrid 3 = AQUAmax™ P1498, Hybrid 4 = 33D49), each plant density level (PD1 = 79,000; and PD2 = 104,000 pl ha⁻¹), and all four N rate levels (Nr1 = 0, Nr2 = 134, Nr3 = 202, and Nr4 = 269 kg N sidedress ha⁻¹) at four growth stages (V5, V10, V15, and R1) during the 2012 growing season at PPAC experimental site. Panel A = hybrid effect (averaged over PDs and N rates), Panel B = PD effect (averaged over hybrids and N rates), Panel C = N rate effect (averaged over hybrids and PDs)

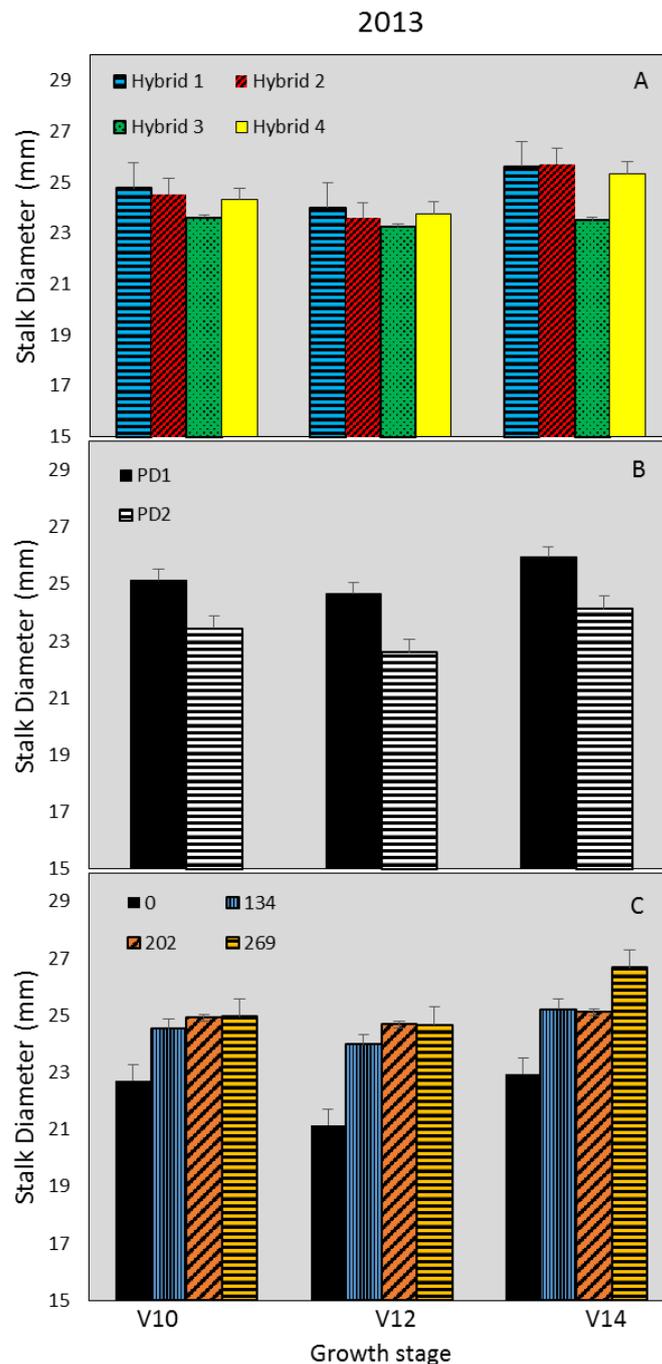


Figure 2.6 - Mean treatment effects on stalk diameter (mm) for all four hybrids (Hybrid 1 = AQUAmax™ P1151, Hybrid 2 = P1162, Hybrid 3 = AQUAmax™ P1498, Hybrid 4 = 33D49), each plant density level (PD1 = 78,000; and PD2 = 99,000 pl ha⁻¹), and all four N rate levels (Nr1 = 0, Nr2 = 134, Nr3 = 202, and Nr4 = 269 kg N sidedress ha⁻¹) at three growth stages (V10, V12, and V14) during the 2013 growing season at PPAC experimental site. Panel A = hybrid effect (averaged over PDs and N rates), Panel B = PD effect (averaged over hybrids and N rates), Panel C = N rate effect (averaged over hybrids and PDs)

2.3.2.2 SPAD Values

Leaf chlorophyll estimates were taken at 7 growth stages in 2012 (from the V10 stage and continuing to R5 stage), but only at 3 growth stages in 2013 (from the V10 to V14) because of time constraints. There were significant treatment effects on SPAD values in 2012 (Figure 2.7; Appendix Table 5.5). The hybrid factor was not significant until the R3 and R4 development stages. Even then, the latter significant differences were quite minor and were only significant between non-drought-tolerant Hybrid 2 and Hybrid 4. The SPAD values averaged greater at PD1 than at PD2 from V10 to R4 stages. The effect of N rate was also minor until late grain fill; the major treatment difference was between plots with and without N (Figure 2.6 C). In 2013 (Figure 2.8; Appendix Table 5.6), SPAD differences were significant for all treatments effects at one or more growth stages. At V12, the drought-tolerant Hybrids (Hybrid 1 and 3) showed higher SPAD values relative to Hybrids 2 and 4. The SPAD values in PD1 were greater than in PD2 for all sampled stages. The lowest N rate 1 (zero N) showed the lowest value for SPAD. This difference was much more apparent in 2013 than in 2012, presumably due to drought conditions and reduced biomass growth and N uptake in 2012 season. Thus, in this study SPAD values were more significant at the later stages. This results are in accordance with the study made by Argenta et al., (2001), they concluded that the SPAD is positively correlated with the N concentration in the leaves of the plants and this is more evident at the later stages. Additionally, according to Piekielek et al. (1995) and Dwyer, Tollenaar, and Houwing (1991), the indirect chlorophyll content in the leaf can be used to predict the nutritional N level in plants, because the correlation with the amount of pigment was positive with the N concentration. Additionally, Lindsey and Thomison (2014) in a field study conducted in 2013 and 2014, in Northwest (NWARS) and Western (WARS) Ohio, evaluating the responses of two drought-tolerant hybrids and two non-tolerant hybrids to sidedress N application rate (0, 67, 134, 202, and 269 kg N ha⁻¹) measured the chlorophyll content (SPAD) at R2 and conclude that the relative chlorophyll content of each hybrid exhibited a similar response to N rate with maximum content occurring at 134 kg N ha⁻¹ at both sites in 2013.

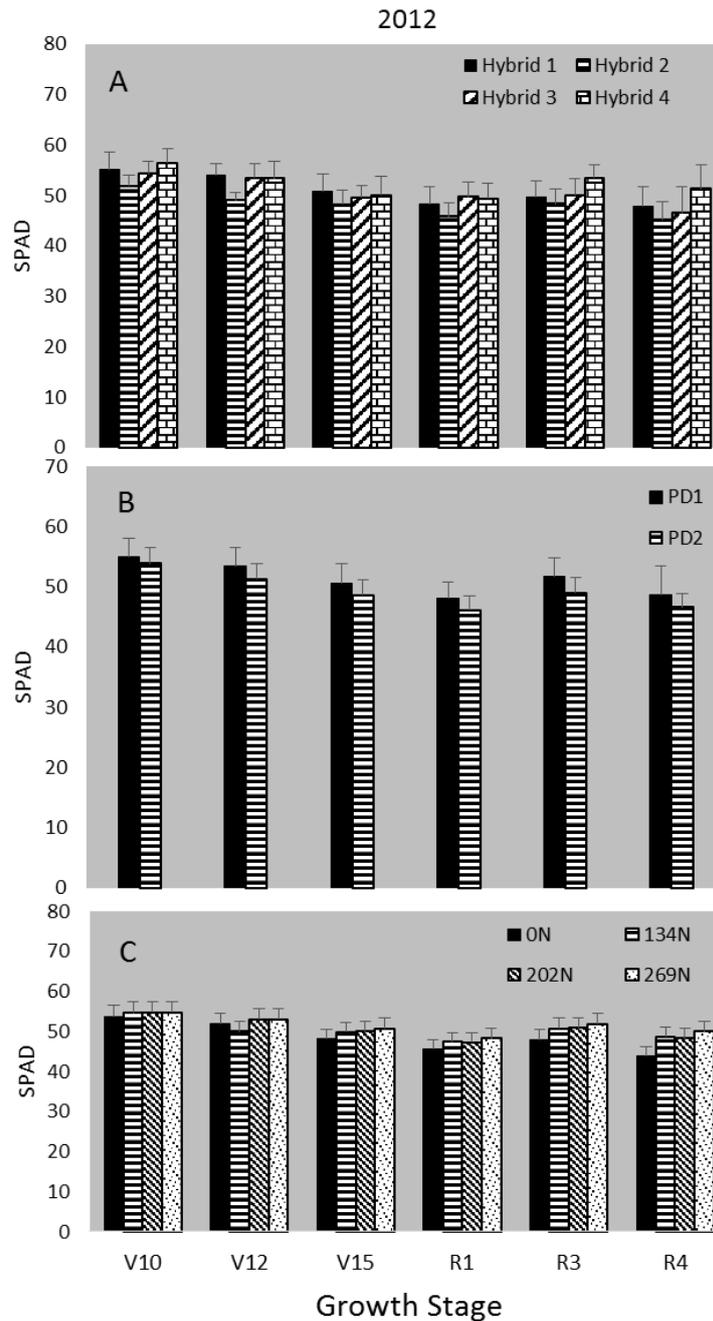


Figure 2.7 - Mean treatment effects on estimated chlorophyll content (SPAD) for all four hybrids (Hybrid 1 = AQUAmax™ P1151, Hybrid 2 = P1162, Hybrid 3 = AQUAmax™ P1498, Hybrid 4 = 33D49), each plant density level (PD1 = 79,000; and PD2 = 104,000 pl ha⁻¹), and all four N rate levels (Nr1 = 0, Nr2 = 134, Nr3 = 202, and Nr4 = 269 kg N sidedress ha⁻¹) at seven growth stages (V10, V12, V15, R1, R3, R4, and R5) at the PPAC experimental site, 2012 growing season. Panel A = hybrid effect (averaged over PDs and N rates), Panel B = PD effect (averaged over hybrids and N rates), Panel C = N rate effect (averaged over hybrids and PDs)

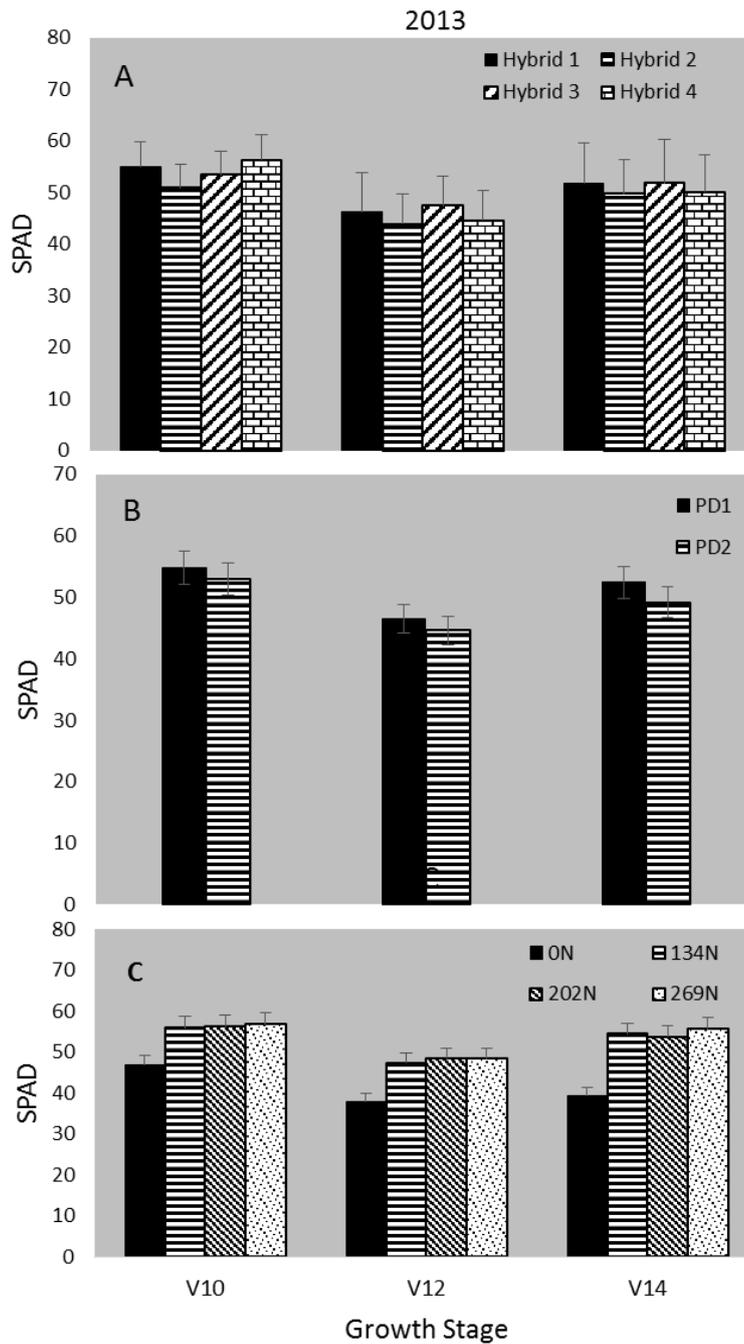


Figure 2.8 - Mean treatment effects on estimated chlorophyll content (SPAD) for all four hybrids (Hybrid 1 = AQUAmax™ P1151, Hybrid 2 = P1162, Hybrid 3 = AQUAmax™ P1498, Hybrid 4 = 33D49), each plant density level (PD1 = 78,000; and PD2 = 99,000 pl ha⁻¹), and all four N rate levels (Nr1 = 0, Nr2 = 134, Nr3 = 202, and Nr4 = 269 kg N sidedress ha⁻¹) at three growth stages (V10, V12, and V14) at PPAC experimental site, 2013 growing season. Panel A = hybrid effect (averaged over PDs and N rates), Panel B = PD effect (averaged over hybrids and N rates), Panel C = N rate effect (averaged over hybrids and PDs)

2.3.3 Anthesis-Silking Interval (ASI)

The first physiological change that affects the number of fertile ears and kernels per plant is the time interval between differentiation of male and female inflorescences (anthesis and silking, respectively). A longer time interval changes the transport rates of phytohormones and carbohydrates within the plant. Thus, ears started later receive smaller amounts of these substances, and are less likely to become functional (SANGOI, 2001). Fewer potential kernels (ovules) become real kernels when silk extrusion is delayed because of lower chances of successful pollination with still-viable pollen.

In 2012, the only single treatment effect on ASI (based on time from 50% pollen shed to 50% silk emergence) was due to hybrid, but a hybrid versus PD interaction was also observed (Table 2.12; Figure 2.2 A, and Appendix Figure 5.1). The lack of a significant N rate effect on ASI in 2012 is not surprising since soil moisture was the major limitation for biomass production (and N uptake) preceding the flowering period. It is also possible that the cumulative effect of the drought during vegetative stages caused minimal moisture availability differences between the PD treatments at flowering. While Hybrids 1 and 3 (drought-tolerant hybrids) did not vary in ASI, there was a significant difference between Hybrid 1 and the pair formed by Hybrids 2 and 4 (non-drought tolerant hybrids). Hybrid 2 experienced the shortest ASI period in comparison with Hybrids 1 and 4. In 2013 (Table 2.12, and Figure 2.2 B), there were ASI differences for both Hybrid and N rate factors. The highest synchrony between anthesis and silk was observed for Hybrid 2, which was different from Hybrids 3 and 4. For the N rate factor, only the control treatment (zero N) was different from the treatments with N applied. Therefore in analyzing the pair of same maturity hybrids we could perceive that Hybrid 3 showed a numeric shorter ASI than Hybrid 4.

2.3.4 Biomass and Plant Nutrient Analysis

2.3.4.1 Total Biomass and Plant Nutrient Uptake and Partitioning

In 2012, the N rate was the sole factor influencing total plant biomass (BM) accumulation at the end of the season, with the major difference being between unfertilized and N fertilized treatments (Table 2.2). The grain and cob fractions were largely influenced by hybrid differences, while the stover partitioning was most significantly influenced by N rate (Nr 1 < Nr 3 and 4). Under more normal weather conditions in 2013 season (Table 2.3), there were significant responses for both hybrid and N rate treatment factors, but not for the PD factor. The grain, cob, and total plant masses responded progressively higher as N rates increased, while the stover

variable did not increase beyond the 134 kg ha⁻¹ N rate. Hybrid 2 achieved the highest total plant biomass; there were no statistical total biomass difference among other hybrids, but only between them and Hybrid 2.

The Grain Harvest Index (GHI) in 2012 (Table 2.2) was affected only by hybrid, there was no difference between the pair formed by Hybrid 1 and 2, but there was a difference between Hybrids 3 and 4 (Hybrid 4 > Hybrid 3). However, a significant factorial (hybrid x PD) was observed for GHI in 2012 (Figure 2.17). Looking at the PD response within the hybrids, there was a significant difference only between Hybrids 1 and 4. The GHI in Hybrid 1 was affected negatively by the higher PD (PD1 > PD 2), but Hybrid 4 was not affected negatively by the higher PD (PD 1 < PD2). For Hybrids in the PD, at PD1 there was no difference between Hybrids 1 and 2, but Hybrid 3 had a lower value than Hybrid 4 for GHI. In analyzing the hybrids in PD2, Hybrid 4 was the greatest, and the drought-tolerant hybrids had lower values than their same maturity pair counterparts. Nevertheless, in the 2013 season (Table 2.3), all treatment factors were significant for GHI; the drought-tolerant hybrids had higher GHI in comparison to the non-drought tolerant hybrids. The GHI was negatively affected by the PD (PD1 > PD2), but positively affected by N rate as highest GHI occurred at the highest N rate (Nr4 > Nr2 > Nr 1). The GHI was higher in 2013 than in 2012, this could happened because the different weather conditions between the seasons, with more rainfall in 2013.

In a similar manner as for plant biomass, the total plant N uptake in the 2012 season was only affected by the N rate (Table 2.4), the greatest value for this variable being for the N fertilized treatments, and there was only a significant difference between the treatments with and without N fertilization. For the grain N content, there were significant effects for hybrid (Hybrid 1 = Hybrid 2; Hybrid 3 < Hybrid 4) and for the N rate factor (Nr 4 > Nr 1).

In the factors evaluated at separate, for stover N content only the N rate factor caused significant differences, in 2012, whereby the N fertilized treatments caused higher stover N than the zero N control. However, there was also a significant interaction between hybrid and PD (Figure 2.11). In the factorial Hybrid x PD, Stover N was higher for Hybrid 1 in PD2 than in PD1. For the other hybrids there was no significant difference in Stover N content between PD treatments. Evaluating the pairs of hybrids in relation to the PD factor, Hybrid 1 did not differ from Hybrid 2, but the drought-tolerant Hybrid 3 did differ from Hybrid 4 (H1=H2; H3 > H4) in the PD2, so the drought-tolerant Hybrid 3 showed a higher value in relation to non-drought tolerant Hybrid 4 for stover N content as PD increased.

In the 2013 season, total whole-plant N uptakes at maturity weren't that different from 2012, but the 2013 grain N content averaged about 28% higher while the 2013 stover N

contents averaged about 41% lower than in 2012 (Tables 2.4 and 2.5), this happened because the climatic difference between the seasons: different weather conditions between these two seasons, with less precipitation and consequently less water availability in the soil in 2012 than in 2013 had influenced the nitrogen partitioning in the plant between the seasons. Therefore, the stover N content was higher in 2012 than in 2013 because the deficiency of water for the process of the N partitioning from the stover to the grains. As consequence of the more normal precipitation in 2013 than in 2012, a higher grain N content was observed. The hybrid and N rate factors resulted in significant differences in grain N content, but there was no difference between the pairs of the same maturity hybrids (Hybrid 1 = Hybrid 2; Hybrid 3 = Hybrid 4). A strong difference for N rate was observed ($Nr1 < Nr2 < Nr3 < Nr4$), Table 2.4, in comparison to 2012. For stover N content and total plant N uptake, the hybrid and N rate factors were also significant. For stover N content the Hybrid 2 was greater than Hybrid 1 and there was no difference between Hybrids 3 and 4, and the higher N rate increased N content in a predictable manner ($Nr1 < Nr2 < Nr3=4$). For total plant N uptake, Hybrids 1 and 2 were greater than Hybrids 3 and 4 (there was no difference between the pairs formed by Hybrids 1 and 2; and by Hybrids 3 and 4), each N rate increment resulted in progressively higher total N uptake.

Unlike BM and N uptake, the P uptake and partitioning in the 2012 season was only affected by the hybrid factor, but not by the N rate (Table 2.6). The stover P content was higher for the Hybrid 3 in comparison to Hybrid 4, and the numeric average for Hybrid 1, stover P content, was higher than Hybrid 2, so in this dry season the drought-tolerant hybrids (AQUAmax) presented higher P concentration in the stover than the non-drought tolerant. Nevertheless, significant interactions of the factorial Hybrid and PD for stover P content (Figure 2.12), and for total plant P uptake (Figure 2.13) were observed. The Hybrid 4 had the higher value for grain P content, in relation to the Hybrid 3 and also to the other hybrids, and the highest total plant P uptake (Hybrid 4 > Hybrid 1 = 2), although Hybrid 4 did not differ significantly from Hybrid 3. In a closer examination of the factorial Hybrid x PD for stover P content (Figure 2.12), there was only a PD difference for Hybrid 4, whereby PD2 negatively affected stover P content in this hybrid. Among hybrids in the same PD, Hybrid 4 had the smallest value for stover P content in the PD2, while no difference among the other hybrids was found. Only Hybrid 3, in the factorial PD versus Hybrid, demonstrated significantly higher total plant P uptake with PD2. For the Hybrid and plant density interaction Hybrid 4 responded with more total plant P uptake than Hybrid 3. There was no difference between Hybrid 1 and 2 in total plant P uptake at either PD. Hybrids 3 and 4 achieved highest total P

uptake in PD2, but there was no difference in total P uptake between hybrid pairs of similar maturity.

In 2013 season (Table 2.7), N rate treatment factor was significant for these plant component P uptake variables; both the grain P content and total plant P uptake were higher in N rate 4 than in N rates 1 and 2. In contrast to 2012, overall P uptake increased incrementally with higher N rates in 2013. However, for stover P content the hybrid and the N rate were significant, Hybrid 2 was greater than Hybrid 1, while Hybrid 3 did not differ from Hybrid 4. Therefore, the only significant difference that was observed was between N fertilized and non-fertilized treatments been the stover P content superior in the zero N treatment.

There was some differences for P uptake and partitioning between the drought and more normal weather seasons. In 2012 (drought season) drought-tolerant hybrids (Hybrids 1 and 3) presented higher values for stover P content than the non-drought tolerant (Hybrids 2 and 4). This factor could be a drought tolerance mechanism because the drought tolerant hybrids have greater area of roots that can explore the soil better and to do the uptake of the P nutrient. The capacity of plants to access P under limiting conditions depends on important adaptive traits (LÓPEZ-BUCIO et al., 2000). A primary adaptation to low P availability involves postembryonic developmental changes in the root system, which are directed toward enhancing P uptake. These include alterations in branching patterns, total root length, root hair elongation, and lateral root formation (BATES; LYNCH, 1996; BORCH et al., 1999).

Plant K uptake values tended to be much lower in the drought year of 2012 (Table 2.8) than in 2013 (Table 2.9). In 2012, the hybrid factor was significant for grain K content, but only Hybrid 3 differed from its same maturity range counterpart (Hybrid 3 < Hybrid 4). For total plant K uptake there was no difference between the hybrids with the similar maturity. The N rate was significant only for stover K content and total plant K uptake (Nr4=3 > Nr1), respectively, in the 2012 season (Table 2.8). In 2013 (Table 2.9), the grain K content and stover K content were affected by the hybrid and N rate treatment factors, but the total plant K uptake was only affected by the N rate. For the grain K content drought-tolerant hybrids did not differ from the drought susceptible hybrids with the same maturity, while for this variable the Nr4 lead to the greatest value (Nr4 > Nr2 > Nr1). For stover K content Hybrid 2 was greater than all others, and the Nr4 lead to the highest value in relation to Nr 1 and 2. The total plant K uptake was greater in Nr4 (Nr4> Nr2-3 > Nr1). Thus, in the normal weather season the non-drought tolerant hybrids presented higher values for total plant K uptake,

however the drought-tolerant hybrids were numerically greater for grain S content than the non-tolerant.

The S partitioning was affected by hybrid and N rate treatments in 2012 (Table 2.10) when the grain S content was highest in Hybrid 4 (Hybrid 4 > Hybrid 3-2 > Hybrid 1). For the total plant S uptake the Hybrid 4 was also the greatest. The N rate affected only stover S content and total plant S uptake (Nr 1 < Nr2-3-4). However, a significant factorial among hybrids versus PD for stover S content was observed (Figure 2.14); only the Hybrid 4 was affected negatively by PD2. For the hybrids analyzed separately in each PD treatment, there was only a significant difference among the hybrids in the PD2 (Hybrid 3 > Hybrid 4) while Hybrid 1 did not differ from Hybrids 2-3). In 2013 (Table 2.11), most of S partitioning variables were affected by hybrid and N rate and a similar range of values was observed as in 2012. In the grain S content only the Hybrid 4 differed from Hybrids 1 and 2, and the Nr 4 had greater grain S than Nr 1 and 2. In the total plant S uptake, Hybrid 2 had the highest value, and there was only difference between N fertilized and non-fertilized treatments (Nr1 < Nr2-3-4). Stover S content was affected only by hybrids with Hybrid 2 being superior to all other hybrids.

As the same way to the stover P content, drought-tolerant Hybrid 3, in the factorial significant for Hybrid x PD, presented higher stover S content than the Hybrid 4, this also could be a droughty tolerance mechanism presented in this AQUAmax hybrid.

In this study we evaluated also micro-nutrients Zn, Fe, Mn and Cu uptake and partitioning as well its harvest indices and efficiencies, therefore the data are only presented in Appendix (Table numbers 5.7, 5.8, 5.9, 5.10, 5.11, 5.12, 5.13, and 5.14), but not discussed.

In summary, BM, and plant nutrient uptake and partitioning (N, P, K, and S) were more affected by N rates than by hybrid or plant density treatment factors. Highest nutrient uptakes were normally observed in N fertilized treatments, and mainly for the highest N rate treatment (Nr 4), in both seasons. In a study of nutrient accumulation and partitioning in maize hybrids under varying PD and N rates, Ciampitti et al. (2013a) reported macro-nutrient uptake to be predominately influenced by N rate, with greater P and K uptake occurring with higher N rates. In the dry weather season (2012), we observed lower values for total biomass and total plant K uptake than in 2013.

All hybrids generally experienced higher BM with N fertilization. In 2012, the overall BM or GY responsive to the N rates was lower than in 2013; and the hybrids differences largely affected the grain and cob fractions more than the stover fraction in 2012. In 2013 the grain, cob, and total plant masses responded progressively higher as N rates increased. Ciampitti

and Vyn (2012), evaluating conventional maize hybrids, found a similar effect. The different results in 2012 can presumably be related to low yields levels that constrained the responsiveness of grain biomass to higher N rates. It is interesting that the total plant N uptake were similar in both seasons, but in 2013 the grain N content was higher and the stover N content was lower than in 2012. Also in 2012 the Hybrid 1 appeared to be least affected by increasing N rates for both biomass and N uptake. Similar to N uptake and BM, the P uptake and partitioning was mostly affected by the hybrids in 2012, while in 2013 both hybrids and N rates influenced the P uptake. While the total plant P and S uptake were similar in both seasons, the total plant K uptake was much higher in the more normal weather conditions season of 2013. Figures 2.27 and 2.28 reveals the relationship between BM and uptake of N, P, K, and S. Regardless of treatment factors, one unique slope fit all points for BM relationships to N, P, K and S uptake ($r^2 = 0.72, 0.58, 0.74, \text{ and } 0.84$, respectively, in 2012; $r^2 = 0.89, 0.28, 0.60, \text{ and } 0.85$, respectively, in 2013). Although BM does affect plant nutrient uptake, it is also true that BM formation can be largely influenced by varying N rates (CIAMPITTI; VYN, 2011). In 2012 (drought season) drought-tolerant hybrids (Hybrids 1 and 3) presented higher values for stover P content than the non-drought tolerant (Hybrids 2 and 4). This factor could be a drought tolerance mechanism, because the drought tolerant can use cumulative nutrient to improve the water conductance in the plant by decreased water potential inside the plant due to higher concentration of nutrient, and also can use the nutrient for the metabolic processes in the plant. However in 2013 the Hybrid factor was not significant factor.

Table 2.2 - Plant biomass (Mg ha⁻¹) of dry mass of grain, cob, and stover components for all maize hybrids (Hybrid 1 = AQUAmaxTM P1151, Hybrid 2 = P1162, Hybrid 3 = AQUAmaxTM P1498, Hybrid 4 = 33D49) grown in two plant densities (PD1 = 79,000, PD2 = 104,000 pl ha⁻¹) and four N rates (Nr1 = 0, Nr2 = 134, Nr3 = 202, and Nr4 = 269 kg ha⁻¹) in 2012

	Grain Weight+ (Mg ha ⁻¹)	Cob Weight (Mg ha ⁻¹)	Stover Weight (Mg ha ⁻¹)	Total Weight (Mg ha ⁻¹)	GHI
Hybrid					
Hyb 1	5.3 b	0.8 b	8.3	14.4	0.36 bc
Hyb 2	6.6 b	1.3 a	7.7	15.5	0.42 ab
Hyb 3	5.7 b	1.3 a	8.9	15.8	0.35 c
Hyb 4	9.0 a	1.3 a	8.2	18.5	0.49 a
PD					
PD 1	6.4	1.2	8.2	15.8	0.40
PD 2	6.9	1.2	8.3	16.4	0.41
Nr					
Nr 1	6.3	1.1	7.2 b	14.6 b	0.43
Nr 2	6.9	1.2	8.3 ab	16.3 ab	0.41
Nr 3	6.6	1.2	8.9 a	16.7 a	0.37
Nr 4	6.9	1.2	8.6 a	16.7 a	0.4
Anova					
Hyb	**	**	ns	ns	**
PD	ns	ns	ns	ns	ns
Nr	ns	ns	**	**	ns
Hyb x PD	ns	ns	*	ns	*
Hyb x Nr	ns	ns	ns	ns	ns
PD x Nr	ns	ns	ns	ns	ns
Hyb x PD x Nr	ns	ns	ns	ns	ns

ns = not significant; *=P<0.05; **=P<0.01; ***=P<0.0001;

+ = based in 3 reps of whole plant biomass sampling

Table 2.3 - Plant biomass (Mg ha⁻¹) of dry matter of grain, cob, and stover components for all maize hybrids (Hybrid 1 = AQUAmax™ P1151, Hybrid 2 = P1162, Hybrid 3 = AQUAmax™ P1498, Hybrid 4 = 33D49) grown at two plant densities (PD1 = 78,000, PD2 = 99,000 pl ha⁻¹) and four N rates (Nr1 = 0, Nr2 = 134, Nr3 = 202, and Nr4 = 269 kg ha⁻¹) in 2013

	Grain Weight+ (Mg ha ⁻¹)	Cob Weight (Mg ha ⁻¹)	Stover Weight (Mg ha ⁻¹)	Total Weight (Mg ha ⁻¹)	GHI
Hybrid					
Hyb 1	12.6 a	1.2 c	7.3 b	20.7 b	0.55 a
Hyb 2	12.0 a	1.6 a	8.2 a	22.0 a	0.51 bc
Hyb 3	11.4 b	1.2 c	7.5 b	20.0 b	0.52 b
Hyb 4	10.7 b	1.4 b	7.6 b	19.6 b	0.50 c
PD					
PD 1	11.7	1.4	7.3 b	20.4	0.52 a
PD 2	11.7	1.3	8.0 a	20.8	0.51 b
Nr					
Nr 1	7.0 c	0.9 c	6.2 b	13.8 c	0.45 c
Nr 2	12.5 b	1.4 b	8.1 a	21.8 b	0.52 b
Nr 3	13.3 ab	1.5 ab	8.2 a	23.1 ab	0.54 ab
Nr 4	13.8 a	1.6 a	8.1 a	23.6 a	0.55 a
Anova					
Hyb	**	**	**	**	**
PD	ns	ns	**	ns	**
Nr	**	**	**	**	**
Hyb x PD	ns	ns	ns	ns	ns
Hyb x Nr	ns	ns	ns	ns	ns
PD x Nr	ns	ns	ns	ns	ns
Hyb x PD x Nr	ns	ns	ns	ns	ns

ns = not significant; *=P<0.05; **=P<0.01; ***=P<0.0001;

+ = based in 3 reps of whole plant biomass sampling

2.3.5 Grain Yield and Its Components

The GY and its components are described in Tables 2.13 and 2.14, for 2012 and 2013, respectively. In 2012, N rate and PD did not significantly affect KN, likely due to more limiting drought conditions around the flowering time. There was only difference between Hybrid 4 and all other (Hybrid 4 (3101) > Hybrid 3 (1819); Hybrid 2 (2188) = 1 (1843)). A significant factorial, hybrid x PD, for KN was observed (Figure 2.9). PD presented only difference for Hybrid 4, this hybrid was not affected by the higher PDs. For the hybrids within the PD, for PD 1 the non-drought tolerant hybrids had greater values of KN than the drought-tolerant, and for PD2, Hybrid 4 had the highest value.

Nevertheless, unlike the KN, the KW in 2012 (Table 2.13) was affected by all treatment factors. The drought-tolerant hybrids showed the highest values for this variable, during this drought weather season. However, the higher PDs negatively affected the KW, the

difference being more than 10 mg of kernel between PD1 and PD2. Among N rate treatments, there was only difference between N fertilized and non-fertilized treatments ($Nr1 < Nr\ 2-3-4$). A significant factorial (hybrid x PD) was also observed (Figure 2.9), KW being higher for Hybrid 4 in the PD1. Looking at the hybrids in the PDs, Hybrid 2 showed the smallest KW value in relation to all other hybrids in PD1. In PD2 the Hybrid 4 was the only one to be negatively affected by the higher PD. Maize final KW is the result of kernel growth during two stages of the grain-filling period, and these stages may experience different resource availability (CIRILO; ANDRADE, 1996). Kernel weight at physiological maturity depends on the potential kernel size established early in grain filling, and the plant capacity to provide assimilates needed to fulfill this potential during grain filling (BORRÁS; WESTGATE, 2006).

Significant differences ($p < 0.05$) in KN and KW also were evident in all three treatment factors (hybrid, plant density and N rate) in 2013 (Table 2.14). Hybrids 1 (P1151) and 2 (P1162) had the highest KW values, but the lowest KN values, while hybrid 4 (33D49) had just the opposite grain components (i.e. highest KN and lowest KW). As expected, KN and KW were higher at the low density and as N rates increased, although the N rate of 269 kg of N did not significantly increase either KN or KW relative to the 202 kg N rate. The higher PDs did not negatively affect this variable, and the higher N rates caused the greatest values for KN; the mean difference between $Nr4$ and $Nr1$ (zero N) was around 2000 kernels m^{-2} .

It is well known that KN is normally the main grain yield component in maize in different environments (TOLLENAAR, 1977), and genotypic variation in KN as drought intensity changes was also documented by Chapuis et al. (2012), and Moradi et al. (2012). Nitrogen stress reduces final grain number (Kernel Number) by increasing kernel abortion (LEMCOFF; LOOMIS, 1986; PEARSON; JACOBS, 1987). Grain sink size is highly associated with kernel number in grain crops, and kernel number is a function of plant dry matter accumulation. In wheat (*Triticum aestivum* L.), kernel number was linearly related to incident solar radiation in the 30 d preceding anthesis across a range of environments (FISCHER, 1985). Andrade et al. (2000) suggest that water and-or N deficiency reduce the carbon availability and dry BM partitioning to the ear during the critical period that determines grain number. Unfavorable environmental conditions can cause a reduction in the kernel number per plant (FISCHER; PALMER, 1984; KINIRY; RITCHIE, 1985).

Overall grain yields were very high in this experiment in 2013. Plot-combine harvested grain yields were statistically different ($P < 0.05$) for hybrid and N rate treatment

factors (Table 2.14); the same treatment factors were also significantly different for other variables: grain N uptake, total plant N uptake, GHI, and NHI. We observed that hybrid P1151 did not differ from P1162, and only differed from P1498 and 33D49. Hybrid 33D49 had the lowest grain yield, and this hybrid was also significantly lower in yield than its comparable-maturity counterpart (P1498). Grain N uptake was similar for hybrid P1151 and P1162, while 33D49 had the lowest grain N uptake. For whole plant N uptake, there was no difference among P1151 and 1162, but both of these 111 CRM hybrids had about 10% more total plant uptake than the 114 CRM hybrids P1498 and 33D49. The early maturing AQUAmax hybrid P1151 had significantly higher grain harvest index and N harvest index than the other 3 hybrids. In 2012, the PD and N rates were significant (Table 2.13), so that GY was negatively affected by the higher PDs, and there was only difference between N fertilized and non-fertilized treatments; GY in Nr1 (zero N) being approximately 10 Mg ha⁻¹ lower than in N fertilized treatments. In contrast to grain yield, the GHI is impartially stable when plant density increased from low PDs to the optimum plant density for grain yield, and the GHI declines when PD increases beyond the optimum PD for GY (TOLLENAR, 2011). However, in the 2013 season, the hybrids and N rates were significant in relation to GY, because there was no difference between Hybrids 1, 2, and 3 but only with the Hybrid 4 (lowest value for this variable). The N rates strongly affected GY, Nr4 being the best one (Nr1 < Nr2 < Nr3 < Nr4). The plant density is one of the cultural practices that most affects GY, due to the small capacity of issuance of fertile tillers culture, its monoecious floral organization and short flowering period (SANGOI, 2001). The use of high densities stimulates apical dominance (SANGOI, 1996). Thus, the plant invests most of available resources in the pollen production and dispersal due to a drop in growth rates and development of ears and stigma, leading to female infertility and asynchrony between pollen shed and silking (Sangoi and Salvador, 1998a). During the drought season, 2012, the highest PD had an influence on GY, but not in 2013. However, the GHI was only affected by the highest PD in the 2013 season, but not in 2012. Argenta et al. (2001), analyzing two single hybrids of corn sown in four row spacing (0.40, 0.60, 0.80 and 1.00 m) and two populations (50,000 and 60,000 plants ha⁻¹), concluded that grain yield was influenced by reducing the spacing between lines and the plant density.

The water stress effects on maize include the visible symptoms of delayed maturity and reduced biomass and crop grain yield. For example, water stress on maize has been shown to reduce plant height (ÇAKIR, 2004), and leaf area index (TRAORE et al., 2000). Grain yield can be reduced by decreasing yield components as for example kernel number and

kernel weight (OTEGUI; ANDRADE; SUERO 1995; PANDEY; MARANVILLE; ADMOU, 2000). Results of older and newer hybrids grown at two PDs showed that grain yield was closely associated with dry matter accumulation (BM) during the grain-filling period (TOLLENAR; LEE, 2011). Lindsey and Thomison (2014) in a field study evaluating the responses of two drought-tolerant hybrids and two non-tolerant hybrids to sidedress N application rate (0, 67, 134, 202, and 269 kg N ha⁻¹) suggested that drought-tolerant maize hybrids exhibit similar N responses to non-tolerant hybrids, and can be managed using existing N recommendations.

2.3.6 Nutrient Harvest Indices

Overall NHI values were much lower in 2012 than in 2013. Unlike for GHI, in 2012, the hybrid and N rate treatment factor were significant for NHI (Table 2.4, Figure 2.15) the drought-tolerant hybrids were inferior in NHI to the non-drought tolerant hybrids (Hybrid1 < Hybrid 2, and Hybrid 3 < Hybrid 4). There was little NHI response to N rate treatments between the N rate 1 in relation to the N rate 3 (Nr1 > Nr3). In 2013 (Table 2.5, Figure 2.16), all treatments factors were significant for NHI; Hybrid 1 had higher NHI than Hybrid 2, and there was no difference between Hybrids 3 and 4. The higher PD (PD2) negatively affected the NHI in 2013, while higher NHI values were observed in the N fertilized treatments (Nr1 < Nr2 < Nr4).

In a similar way for the GHI in 2012, in the PHI only the hybrid factor was significant (Table 2.6, Figure 2.16); the non-drought tolerant hybrids achieved higher PHI values than the drought-tolerant hybrids (Hybrid 1 < 2; Hybrid 3 < 4). . However, in 2013 only the N rate factor was significant for PHI (Table 2.6), the higher value for that variable was for Nr4 (Nr4 > Nr2-3 > Nr1).

Hybrid treatments, but not PD or N rate treatments, significantly affected KHI in 2012 (Table 2.8), Hybrid 4 had higher KHI than Hybrids 1 and 2, but no difference was found between the same maturity hybrids. In 2013 (Table 2.9), the KHI was affected by both hybrid and N rate treatments, but the hybrids with the same maturity did not differ (Hybrid 1 > Hybrid 3 = Hybrid 4) and only a KHI difference between treatments with and without N was observed (Nr1 < Nr2=3=4).

The SHI in 2012 was affected by the hybrid and N rate treatments (Table 2.10), the drought-tolerant hybrids had lower values for this variable (Hybrid 1 < 2; Hybrid 3 < 4), and Hybrid 4 had higher SHI than all other hybrids while Nr1 greater than Nr3 and Nr4. In 2013,

the SHI was also affected by all three treatment factors (Table 2.11), SHI was negatively affected by the higher PD ($PD1 > PD2$), there was no difference among Hybrids 1 and 2, only between Hybrids 3 and 4 since the Hybrid 3 superior in SHI than the Hybrid 4. The highest value for SHI was for Nr 4 ($Nr4 > Nr2-3 > Nr1$).

In 2013 season, the majority of the macronutrient harvest indices were higher than those observed in 2012. For example, Hybrids 1, 2 and 3 had an incremental gain of approximately 0.20 in the NHI between 2012 and 2013. Only the KHI were similar in both seasons. The PHI was lower than normal in 2012, but also significantly lower in drought-tolerant hybrids than in the two more drought susceptible hybrids.

2.3.7 Nutrient Internal Efficiencies

In 2012, the hybrids and N rate treatment factors were significant for Nitrogen Internal Efficiency, NIE ($Hybrid\ 3 < 4$; $Hybrid\ 1 = Hybrid\ 2$) and N rate 1 was the greatest among all N rates (Table 2.4). A positive factorial, hybrid x PD, for NIE was observed (Figure 2.18). When we evaluated the PD within hybrids, Hybrid 4 had a higher value for NIE in the PD2. While for the other hybrids, Hybrid 4 was greater than Hybrid 3 in the PD 1, and Hybrid 4 responded better in the PD2 than the other hybrids. For NRE only the Hybrids caused significant differences ($Hybrid\ 4 > 3$; and $Hybrid\ 1 = 2$). There was no significant treatment factors for NUE.

In the 2013 season (Table 2.5), only the N rate treatment caused difference for NIE ($Nr1-2 > Nr4$), and NRE ($Nr2 > Nr3-4$). However, hybrid and N rate treatment factors both caused differences for NUE; Hybrid 1 had 6.7% NUE than Hybrid 2 but no NUE differences were observed between Hybrids 3 and 4 ($Hybrid\ 1 > Hybrid\ 2, 3\ and\ 4$) and ($Nr2 > Nr3 > Nr4$).

The NRE, NIE, and NUE values, when significant for N rate, were greater for low N rate treatments for both seasons. In the more normal weather season the drought-tolerant hybrid (Hybrid 1) resulted in the highest value for NUE.

In 2012, PIE was affected only by the hybrid treatment factor ($Hybrid\ 2=4 > Hybrid\ 1 > Hybrid\ 3$) and the drought-tolerant hybrids were inferior to the non-drought tolerant hybrids for this variable (Table 2.6). A significant interaction between hybrid and PD was observed in 2012 (Figure 2.19). In analyzing the PD within the hybrids, there were PD differences for the Hybrids 1 and 4; the PD2 negatively affected the PIE in the Hybrid 1, but positively in the Hybrid 4. Looking at the Hybrids in the PDs, Hybrid 3 had the smallest value at PD1, and

also Hybrid 1 was affected negatively by the PD2. In the 2013 season the PIE was affected only by the N rate ($Nr1 < Nr2=3=4$), Table 2.7.

Both hybrid and N rate treatment factors were significant for KIE in 2012. The drought-tolerant hybrids presented the lower values (Hybrid 2 = 4 > Hybrid 1=3; and $Nr1=2=4 > Nr3$), Table 2.8. In 2013, KIE was higher in the N fertilized treatments ($Nr1 < Nr2=3=4$) but no hybrid or PD treatment effects were significant (Table 2.9).

Therefore, the SIE in 2012 (Table 2.10) was affected only by hybrid, and the more drought susceptible hybrids had superior values for the SIE in 2012. However, in 2013 (Table 2.11), in a similar way PIE, the N rate was the only significant factor. The simple presence of N fertilization resulted in higher SIE ($Nr 2-4 > Nr1$).

2.3.8 Nutrient ratios

The N/P ratio was affected by hybrid and N rate (Nr) in 2012, but only by Nr in the 2013 season (Table 2.12). In 2012, the Hybrids 2 and 3 had the greatest values for the N/P ratio, and the Nr1 presented the smallest value for this variable in both seasons. Significant factorials of Hybrid x PD (Figure 2.22), and Hybrid x Nr were observed (Figure 2.23). In the factorial hybrid x PD, looking at the PD within the hybrids, there was only difference for the Hybrid 2, and the N/P ratio increased in the higher plant density. Analyzing the hybrids within the PD, Hybrids 1 and 2 were the greatest in the PD1. Therefore, in PD 2, the Hybrid 2 was the less negatively affected by the higher PD (Hybrid 2 > Hybrid 1; Hybrid 4 > Hybrid 3). For the factorial hybrid x Nr, for the Hybrids 1 and 2 most of the N rates only differed from zero N, while for Hybrid 3 the N rate 3 lead to the greatest value for N/P, and in the Hybrid 4 the N/P ratio was higher at the higher N rates. Looking at the hybrids within the N rates, we noted that the Hybrids 1 and 2 were individually greater in most of them, but in the Nr4 also Hybrid 4 responded with a higher value for N/P, only Hybrid 3 had the smallest value.

The N/K ratio was affected by hybrid and Nr in both years. In a similar way as the N/P ratio for the Hybrids 1 and 2, in 2012, these hybrids were again greater than Hybrids 3 and 4, and the Nr1 (zero N) triggered the smallest value. Also, like for N/P, significant factorials were observed for ratio N/K between Hybrid x PD (Figure 2.25), and Hybrid x Nr (Figure 2.26). In addition, there was a significant factorial between all three treatment factors (Hybrid x PD x Nr), Figure 2.24. For the factorial hybrid x PD, looking at the PD within the hybrids, there was only difference between the two PD for the Hybrid 3, the N/K was higher in PD 1, while analyzing the hybrids within the PDs, we distinguished that the Hybrids 1 and 2 had higher N/K ratios in both PDs.. Then, for the factorial hybrid x Nr, the Nr within the hybrids,

presented more differences between N fertilized and non-fertilized treatments, however for the Hybrid 4 the highest Nr did not differ from the zero N fertilization. In examining the hybrids within the N rates we could also see that Hybrids 1 and 2 had higher N/K ratios in the majority of the N rates.

Nevertheless in 2013, Hybrids 1 did not differ in its N/K ratio from Hybrid 2 and Hybrid 4 was greater than Hybrid 3, and the higher N rates caused the greatest N/K values ($Nr_{3-4} > Nr_2 > Nr_1$). However, the ratio N/S was affected by hybrid only in 2012, not in 2013, by PD only in 2012, and by Nr in both seasons. Similar to N/P and N/K ratio results in 2012, Hybrids 1 and 2 resulted in higher N/S (although there was no difference between the similar maturity hybrids). The N/S value was not negatively affected by higher PD, and the higher Nr triggered the greater value ($Nr_{3=4} > Nr_2 > Nr_1$). Nonetheless, in 2013 only the Nr was significant for N/S, was the greatest, and an increase in this variable was observed with the increase of N rates ($Nr_4 > Nr_3 > Nr_2 > Nr_1$). In summary, there was only difference between the same maturity hybrids for the pair formed by Hybrid 3 and Hybrid 4 for the ratio N/K in 2013, Hybrid 3 presented a lower value than Hybrid 4 for this variable.

2.3.9 Regression analysis

The total plant biomass (BM) had the highest r^2 value in relation to total plant S uptake, in the 2012 season (Figure 2.27). The weakest correlation between BM and nutrient uptake was with total plant P uptake. The total plant N uptake was the third highest r^2 for BM x nutrient uptake (total plant S uptake > total plant K uptake > total plant N uptake > total plant P uptake). In 2013 (Figure 2.28), the N uptake had the highest regression relationship with BM. However, the total plant P uptake, as in 2012, had the weakest correlation values (total plant N uptake > total plant S uptake > total plant K uptake > total plant P uptake).

In the normal weather season of 2013 the plant N uptake had a higher influence in the total plant biomass. However, in the dry conditions season of 2012, the total plant S uptake resulted in the highest regression between the BM and nutrient uptake. The total plant P uptake was the macro-nutrient least related with the BM for all hybrids averaged across both PD and all N rates. As a result, a rather substantial positive influence in relation to the amount of nutrient uptake and BM was observed, mainly for N, K, and S, in both seasons.

2.4 Conclusions

In the dry season, drought-tolerant hybrids (Hybrids 1 and 3) presented higher values for stover P content than the non-drought tolerant (Hybrids 2 and 4). The drought-tolerant

Hybrid 3, in the factorial significant for Hybrid x PD, presented higher stover S content than the Hybrid 4. Demonstrating that these drought tolerant hybrids have greater root area than the non-drought tolerant.

In 2013 season, the majority of the macronutrient harvest indices were higher than those observed in 2012. Only the KHI were similar in both seasons. The PHI was lower than normal in 2012, but also significantly lower in drought-tolerant hybrids than in the two more drought susceptible hybrids.

All hybrids, whether labeled as more drought tolerant or less so, responded similarly in grain yield to plant density and N rate treatments in the dry or more normal weather year. AQUAmax hybrids (drought tolerant) did not demonstrate improvement in grain yield or more yield stability than non-AQUAmax (non-drought tolerant). The drought-tolerant Hybrid 1 (P1151) generally displayed a longer anthesis to silking interval than comparable-maturity Hybrid P1162 (in 2013 season). AQUAmax Hybrid P1498 generally showed a slightly earlier flowering, and a shorter anthesis-to-silking interval, than comparable-maturity hybrid 33D49 in both seasons (2012 and 2013). Hybrid 1498 also had consistently lower kernel numbers but higher final kernel weights than 33D49 in both years.

Achieving shorter periods between anthesis and silking and accumulation of more nutrients in the stover, in the dry season, could be some of the mechanisms used by drought-tolerant hybrids in achieving greater plant BM production and possible higher GY.

There was no evidence that AQUAmax hybrids were any different than non-AQUAmax hybrids in their response to N fertilizers or in their N use efficiencies. Thus it is unlikely that N fertilizer management should change when AQUAmax hybrids are grown. Certainly there is no evidence that optimum N fertilizer rates would be any lower for these AQUAmax hybrids.

Table 2.4 - Nitrogen partitioning (kg ha^{-1} dry weight) into grain, cob, and stover components, nitrogen harvest index (NHI), nitrogen use efficiency (NUE) $\text{kg grain (kg N}^{-1})$ (ΔGY per unit N applied – relative to Nr1, control treatment), nitrogen recovery efficiency (NRE), nitrogen internal efficiency (NIE) at physiological maturity for all maize hybrids (Hybrid 1 = AQUAmaxTM P1151, Hybrid 2 = P1162, Hybrid 3 = AQUAmaxTM P1498, Hybrid 4 = 33D49) grown at two plant densities (PD1 = 79,000, PD2 = 104,000 pl ha^{-1}) and four N rates (Nr1 = 0, Nr2 = 134, Nr3 = 202, and Nr4 = 269 kg ha^{-1}) in 2012

	Grain N (kg ha^{-1})	Cob N (kg ha^{-1})	Stover N (kg ha^{-1})	Total N (kg ha^{-1})	NHI	NUE	NRE	NIE
Hybrid								
Hyb 1	77.6 b	6.6	108.2	192.3	0.40 c	3.2	0.18 c	28.4 b
Hyb 2	96.9 ab	8.0	92.4	197.3	0.49 b	8.8	0.34 bc	33.8 b
Hyb 3	86.2 b	8.5	103.3	198.1	0.44 bc	3.9	0.35 b	29.0 b
Hyb 4	127.0 a	8.4	90.0	225.4	0.56 a	6.7	0.49 a	40.9 a
PD								
PD 1	94.8	6.8	97.0	199.6	0.47	5.0	0.30	32.4
PD 2	99.0	8.9	99.0	207.0	0.47	6.3	0.43	33.5
Nr								
Nr 1	81.3 b	6.9	70.5 c	158.7 b	0.51 a	-	-	39.8 a
Nr 2	100.3 ab	7.6	98.9 b	206.8 a	0.48 ab	7.4	0.39	33.6 b
Nr 3	100.5 ab	8.0	116.2 a	224.6 a	0.43 b	5.1	0.36	28.2 b
Nr 4	105.7 a	8.9	108.3 ab	222.9 a	0.47 ab	4.3	0.26	30.3 b
Anova								
Hyb	*	na	ns	ns	**	ns	**	**
PD	ns	na	ns	ns	ns	ns	ns	ns
Nr	*	na	***	***	*	ns	ns	***
Hyb x PD	ns	na	*	ns	ns	ns	ns	*
Hyb x Nr	ns	na	ns	ns	ns	ns	ns	ns
PD x Nr	ns	na	ns	ns	ns	ns	ns	ns
Hyb x PD x Nr	ns	na	ns	ns	ns	ns	ns	ns

ns = not significant; na = not available; *= $P < 0.05$; **= $P < 0.01$; ***= $P < 0.0001$.

Cob nutrient concentration for the same treatments in reps 2 and 3 was estimated from rep 1 data but adjusted for each individual plot cob weight so statistical analysis was not possible

Table 2.5 - Nitrogen partitioning (kg ha⁻¹ dry weight) into grain, cob, and stover components, nitrogen harvest index (NHI), nitrogen use efficiency (NUE) kg grain (kg N⁻¹) (Δ GY per unit N applied – relative to Nr1 control treatment), nitrogen recovery efficiency (NRE), nitrogen internal efficiency (NIE) at physiological maturity for all maize hybrids (Hybrid 1 = AQUAmaxTM P1151, Hybrid 2 = P1162, Hybrid 3 = AQUAmaxTM P1498, Hybrid 4 = 33D49) grown at two plant densities (PD1 = 78,000, PD2 = 99,000 pl ha⁻¹) and four N rates (Nr1 = 0, Nr2 = 134, Nr3 = 202, and Nr4 = 269 kg ha⁻¹) in 2013

	Grain N	Cob N	Stover N	Total N	NHI	NUE	NRE	NIE
	(kg ha ⁻¹)							
Hybrid								
Hyb 1	135.8 a	8.4	52.8 b	197.0 a	0.67 a	40.7 a	0.55	68.0
Hyb 2	128.9 ab	7.8	65.2 a	201.9 a	0.62 b	34.0 b	0.58	66.8
Hyb 3	119.6 bc	8.6	53.4 b	181.5 b	0.64 b	29.6 b	0.50	69.9
Hyb 4	113.9 c	8.9	55.1 b	178.0 b	0.62 b	33.4 b	0.55	66.4
PD								
PD 1	125.1	8.0	54.0	187.1	0.65 a	34.8	0.51	68.8
PD 2	124.0	8.8	59.3	192.1	0.62b	34.3	0.58	66.7
Nr								
Nr 1	60.4 d	6.0	43.1 c	109.5 d	0.54 c	-	-	69.0 a
Nr 2	125.2 c	9.2	57.2 b	191.7 c	0.65 b	43.3 a	0.63 a	70.8 a
Nr 3	146.4 b	8.9	61.3 a	216.6 b	0.67 ab	33.7 b	0.53 b	67.5 ab
Nr 4	166.2 a	9.5	65.0 a	240.7 a	0.69 a	27.8 c	0.49 b	63.5 b
Anova								
Hyb	**	na	*	*	**	**	ns	ns
PD	ns	na	ns	ns	*	ns	ns	ns
Nr	**	na	**	**	**	**	*	*
Hyb x								
PD	ns	na	ns	ns	ns	ns	ns	ns
Hyb xNr								
PD x Nr	ns	na	ns	ns	ns	ns	ns	ns
Hyb x								
PD x Nr	ns	na	ns	ns	ns	ns	ns	ns

ns = not significant; na = not available; *=P<0.05; **=P<0.01; ***=P<0.0001.

Cob nutrient concentration for the same treatment in reps 2 and 3 was estimated from rep 1 data but adjusted for each individual plot cob weight so statistical analysis was not possible.

Table 2.6 - Phosphorus partitioning (kg ha⁻¹ dry weight) into grain, cob, and stover components, phosphorus harvest index (PHI) and phosphorus internal efficiency (PIE) at physiological maturity for all maize hybrids (Hybrid 1 = AQUAmax™ P1151, Hybrid 2 = P1162, Hybrid 3 = AQUAmax™ P1498, Hybrid 4 = 33D49) grown at two plant densities (PD1 = 79,000, PD2 = 104,000 pl ha⁻¹) and four N rates (Nr1 = 0, Nr2 = 134, Nr3 = 202, and Nr4 = 269 kg ha⁻¹) in 2012

	Grain P (kg ha ⁻¹)	Cob P (kg ha ⁻¹)	Stover P (kg ha ⁻¹)	Total P (kg ha ⁻¹)	PHI	PIE
Hybrid						
Hyb 1	13.6 c	0.9	17.0 a	31.5 b	0.42 c	170.6 b
Hyb 2	16.8 c	1.1	13.2 ab	31.2 b	0.53 b	219.2 a
Hyb 3	22.5 b	1.4	17.0 a	40.8 ab	0.54 b	137.8 c
Hyb 4	32.1 a	0.8	11.0 b	44.0 a	0.73 a	210.2 a
PD						
PD 1	20.5	0.8	15.1	36.4	0.54	180.4
PD 2	22	1.3	14	37.3	0.56	188.4
Nr						
Nr 1	20.1	0.9	13.2	34.2	0.57	188
Nr 2	21	1.2	14.4	36.6	0.56	202.3
Nr 3	21.8	1	15.7	38.5	0.52	167.3
Nr 4	22.1	1.2	14.9	38.3	0.56	180.2
Anova						
Hyb	***	na	*	**	***	**
PD	ns	na	ns	ns	ns	ns
Nr	ns	na	ns	ns	ns	ns
Hyb x PD	ns	na	*	**	ns	**
Hyb xNr	ns	na	ns	ns	ns	ns
PD x Nr	ns	na	ns	ns	ns	ns
Hyb x PD x Nr	ns	na	ns	ns	ns	ns

ns = not significant; na = not available; *=P<0.05; **=P<0.01; ***=P<0.0001

Cob nutrient concentration for the same treatments in reps 2 and 3 was estimated from rep 1 data but adjusted for each individual plot cob weight so statistical analysis was not possible

Table 2.7 - Phosphorus partitioning (kg ha^{-1} dry weight) into grain, cob, and stover components, phosphorus harvest index (PHI) and phosphorus internal efficiency (PIE) at physiological maturity for all maize hybrids (Hybrid 1 = AQUAmax™ P1151, Hybrid 2 = P1162, Hybrid 3 = AQUAmax™ P1498, Hybrid 4 = 33D49) grown at two plant densities (PD1 = 78,000, PD2 = 99,000 pl ha^{-1}) and four N rates (Nr1 = 0, Nr2 = 134, Nr3 = 202, and Nr4 = 269 kg ha^{-1}) in 2013

	Grain P (kg ha^{-1})	Cob P (kg ha^{-1})	Stover P (kg ha^{-1})	Total P (kg ha^{-1})	PHI	PIE
Hybrid						
Hyb 1	34.6	0.46	5.3 b	40.4	0.83	347.1
Hyb 2	32.8	0.42	8.0 a	41.6	0.8	332.5
Hyb 3	30.9	0.52	5.2 b	36.6	0.82	356.2
Hyb 4	29.5	0.61	5.5 b	35.6	0.8	339.7
PD						
PD 1	32.4	0.5	5.8	38.2	0.82	338.4
PD 2	31.4	0.5	6.2	38.2	0.81	349.6
Nr						
Nr 1	20.3 c	0.5	10.9 a	31.6 c	0.63 c	241.8 b
Nr 2	31.0 b	0.6	4.9 b	36.5 bc	0.84 b	387.6 a
Nr 3	35.3 ab	0.4	4.6 b	40.4 ab	0.87 b	376.6 a
Nr 4	40.8 a	0.4	3.7 b	44.9 a	0.90 a	364.6 a
Anova						
Hyb	ns	na	**	ns	ns	ns
PD	ns	na	ns	ns	ns	ns
Nr	**	na	**	**	**	**
Hyb x PD	ns	na	ns	ns	ns	ns
Hyb x Nr	ns	na	ns	ns	**	ns
PD x Nr	ns	na	ns	ns	ns	ns
Hyb x PD x Nr	ns	na	ns	ns	ns	ns

ns = not significant; na = not available; *= $P < 0.05$; **= $P < 0.01$; ***= $P < 0.0001$

Cob nutrient concentration for the same treatments in reps 2 and 3 was estimated from rep 1 data but adjusted for each individual plot cob weight so statistical analysis was not possible

Table 2.8 - Potassium partitioning (kg ha⁻¹ dry weight) into grain, cob, and stover components, potassium harvest index (KHI), and potassium internal efficiency (KIE) at physiological maturity for all maize hybrids (Hybrid 1 = AQUAmax™ P1151, Hybrid 2 = P1162, Hybrid 3 = AQUAmax™ P1498, and Hybrid 4 = 33D49) grown at two plant densities (PD1 = 79,000, PD2 = 104,000 pl ha⁻¹) and four N rates (Nr1 = 0, Nr2 = 134, Nr3 = 202, and Nr4 = 269 kg ha⁻¹) in 2012

	Grain K (kg ha ⁻¹)	Cob K (kg ha ⁻¹)	Stover K (kg ha ⁻¹)	Total K (kg ha ⁻¹)	KHI	KIE
Hybrid						
Hyb 1	18.9 b	6.9	84	109.7 b	0.17 b	49.7 b
Hyb 2	21.5 b	8.4	80	110.0 b	0.20 b	61.4 a
Hyb 3	27.1 b	9.3	91.6	128.0 ab	0.21 ab	44.4 b
Hyb 4	38.2 a	16.3	99.5	154.1 a	0.25 a	60.2 a
PD						
PD 1	25.3	9.2	87	121.4	0.21	53.4
PD 2	27.6	11.3	90.6	129.5	0.21	54.4
Nr						
Nr 1	25.2	9.8	75.0 b	110.0 b	0.23	58.1 a
Nr 2	26.4	10.5	86.6 ab	123.5 ab	0.21	58.8 a
Nr 3	26.7	10	99.9 a	136.6 a	0.19	46.7 b
Nr 4	27.4	10.5	93.6 a	131.6 a	0.21	52.1 a
Anova						
Hyb	**	na	ns	*	**	**
PD	ns	na	ns	ns	ns	ns
Nr	ns	na	ns	**	ns	**
Hyb x PD	ns	na	ns	ns	ns	*
Hyb x Nr	ns	na	ns	ns	ns	*
PD x Nr	ns	na	ns	ns	ns	ns
Hyb x PD x Nr	ns	na	ns	ns	ns	ns

ns = not significant; na = not available; *=P<0.05; **=P<0.01; ***=P<0.0001

Cob nutrient concentration for the same treatments in reps 2 and 3 was estimated from rep 1 data but adjusted for each individual plot cob weight so statistical analysis was not possible

Table 2.9 - Potassium partitioning (kg ha⁻¹ dry weight) into grain, cob, and stover components, potassium harvest index (KHI), and potassium internal efficiency (KIE) at physiological maturity for all maize hybrids (Hybrid 1 = AQUAmax™ P1151, Hybrid 2 = P1162, Hybrid 3 = AQUAmax™ P1498, and Hybrid 4 = 33D49) grown at two plant densities (PD1 = 78,000, PD2 = 99,000 pl ha⁻¹) and four N rates (Nr1 = 0, Nr2 = 134, Nr3 = 202, and Nr4 = 269 kg ha⁻¹) in 2013

	Grain K (kg ha ⁻¹)	Cob K (kg ha ⁻¹)	Stover K (kg ha ⁻¹)	Total K (kg ha ⁻¹)	KHI	KIE
Hybrid						
Hyb 1	47.5 a	10.3	98.4 b	156.3	0.30 a	85.2
Hyb 2	46.0 a	10.2	115.8 a	172.4	0.27 ab	77.8
Hyb 3	43.5 ab	11.6	108.5 b	163.7	0.26 b	77.3
Hyb 4	37.0 b	16.6	96.4 b	150.1	0.24 b	77.3
PD						
PD 1	44.2	11.7	101.4	157.3	0.28	80.3
PD 2	43	12.8	108.1	163.7	0.26	78.5
Nr						
Nr 1	29.1 c	10.2	87.5 c	125.9 c	0.23 b	59.3 b
Nr 2	43.8 b	13.7	104.5 b	162.0 b	0.27 a	84.8 a
Nr 3	47.6 ab	12.8	107.2 ab	167.7 b	0.28 a	88.8 a
Nr 4	53.6 a	12.2	119.8 a	185.6 a	0.29 a	83.4 a
Anova						
Hyb	*	na	*	ns	**	ns
PD	ns	na	ns	ns	ns	ns
Nr	*	na	*	**	**	**
Hyb x PD	ns	na	ns	ns	ns	ns
Hyb x Nr	ns	na	ns	ns	ns	ns
PD x Nr	ns	na	ns	ns	ns	ns
Hyb x PD x Nr	ns	na	ns	ns	ns	ns

ns = not significant; na = not available; *=P<0.05; **=P<0.01; ***=P<0.0001

Cob nutrient concentration for the same treatments reps 2 and 3 was estimated from rep 1 data but adjusted for each individual plot cob weight so statistical analysis was not possible

Table 2.10 - Sulfur partitioning (kg ha⁻¹ dry weight) into grain, cob, and stover components, sulfur harvest index (SHI), and sulfur internal efficiency (SIE) at physiological maturity for all maize hybrids (Hybrid 1 = AQUAmax™ P1151, Hybrid 2 = P1162, Hybrid 3 = AQUAmax™ P1498, Hybrid 4 = 33D49) grown at two plant densities (PD1 = 79,000, PD2 = 104,000 pl ha⁻¹) and four N rates (Nr1 = 0, Nr2 = 134, Nr3 = 202, and Nr4 = 269 kg ha⁻¹) in 2012

	Grain S (kg ha ⁻¹)	Cob S (kg ha ⁻¹)	Stover S (kg ha ⁻¹)	Total S (kg ha ⁻¹)	SHI	SIE
Hybrid						
Hyb 1	5.6 c	0.4	7.3	13.1 c	0.42 c	386.0 b
Hyb 2	6.9 b	0.5	6.1	14.2 bc	0.50 b	493.2 a
Hyb 3	6.8 b	0.7	7.5	14.8 b	0.45 bc	374.9 b
Hyb 4	9.6 a	0.5	6.7	16.7 a	0.57 a	538.4 a
PD						
PD 1	7.2	0.4	7	14.6	0.5	436.2
PD 2	7.2	0.6	6.8	14.7	0.5	460.2
Nr						
Nr 1	7.1	0.5	5.7 b	13.3 b	0.53 a	471.8
Nr 2	7.5	0.6	7.0 a	15.1 a	0.49 ab	459.6
Nr 3	7.2	0.5	7.7 a	15.4 a	0.45 bc	409.4
Nr 4	7.2	0.6	7.1 a	15.0 a	0.47 b	451.9
Anova						
Hyb	**	na	ns	**	**	**
PD	ns	na	ns	ns	ns	ns
Nr	ns	na	**	*	*	ns
Hyb x PD	ns	na	*	ns	ns	**
Hyb xNr	ns	na	ns	ns	ns	ns
PD x Nr	ns	na	ns	ns	ns	ns
Hyb x PD x Nr	ns	na	ns	ns	ns	ns

ns = not significant; na = not available; *=P<0.05; **=P<0.01; ***=P<0.0001

Cob nutrient concentration for the same treatments in reps 2 and 3 was estimated from rep 1 data but adjusted for each individual plot cob weight so statistical analysis was not possible

Table 2.11 - Sulfur partitioning (kg ha⁻¹ dry weight) into grain, cob, and stover components, sulfur harvest index (SHI), and sulfur internal efficiency (SIE) at physiological maturity for all maize hybrids (Hybrid 1 = AQUAmaxTM P1151, Hybrid 2 = P1162, Hybrid 3 = AQUAmaxTM P1498, Hybrid 4 = 33D49) grown at two plant densities (PD1 = 78,000, PD2 = 99,000 pl ha⁻¹) and four N rates (Nr1 = 0, Nr2 = 134, Nr3 = 202, and Nr4 = 269 kg ha⁻¹) in 2013

	Grain S (kg ha ⁻¹)	Cob S (kg ha ⁻¹)	Stover S (kg ha ⁻¹)	Total S (kg ha ⁻¹)	SHI	SIE
Hybrid						
Hyb 1	9.1 a	0.4	4.0 b	13.5 b	0.66 a	974.2
Hyb 2	9.7 a	0.3	4.8 a	14.8 a	0.64 a	884.2
Hyb 3	8.9 ab	0.4	4.0 b	13.8 b	0.65 a	943.7
Hyb 4	8.1 b	0.4	4.0 b	12.5 c	0.63 b	918.6
PD						
PD 1	9.1	0.4	4	13.5	0.66 a	928.9
PD 2	8.9	0.4	4.4	13.6	0.64 b	932.4
Nr						
Nr 1	5.3 c	0.3	4	9.6 b	0.55 c	773.1 b
Nr 2	9.5 b	0.4	4.3	14.2 a	0.67 b	919.7 a
Nr 3	10.3 ab	0.4	4.4	15.1 a	0.68 b	892.4 ab
Nr 4	10.7 a	0.4	4.2	15.3 a	0.70 a	1007.0 a
Anova						
Hyb	**	na	**	**	*	ns
PD	ns	na	ns	ns	*	ns
Nr	**	na	ns	**	*	**
Hyb x PD	ns	na	ns	ns	ns	ns
Hyb xNr	ns	na	ns	ns	ns	ns
PD x Nr	ns	na	ns	ns	ns	ns
Hyb x PD x Nr	ns	na	ns	ns	ns	ns

ns = not significant; na = not available; *= $P < 0.05$; **= $P < 0.01$; ***= $P < 0.0001$

Cob nutrient concentration for the same treatments in reps 2 and 3 was estimated from rep 1 data but adjusted for each individual plot cob weight so statistical analysis was not possible

Table 2.12 - Plant nitrogen ratios to phosphorus (N/P), potassium (N/K), and sulfur (N/S), and anthesis-silking interval (ASI) at physiological maturity for all maize hybrids (Hybrid 1 = AQUAmax™ P1151, Hybrid 2 = P1162, Hybrid 3 = AQUAmax™ P1498, Hybrid 4 = 33D49, and Hybrid 5 = P1184) grown at two plant densities (PD1=79,000, PD2=104,000 pl ha⁻¹) and (PD1 = 78,000, PD2 = 99,000 pl ha⁻¹) 2012 and 2013 seasons, respectively, and four N rates (Nr1 = 0, Nr2 = 134, Nr3 = 202, and Nr4 = 269 kg ha⁻¹) in 2012 and 2013

	Ratio N/P		Ratio N/K		Ratio N/S		ASI R1 (days)	
	2012	2013	2012	2013	2012	2013	2012	2013
Hybrid								
Hyb 1	6.23 a	5.06	1.79 a	1.25 a	14.26 a	14.34	5.6 a	0.5 bc
Hyb 2	6.65 a	5.09	1.81 a	1.16 ab	14.67 a	13.4	3.5 b	0.29 c
Hyb 3	4.80 b	5.12	1.56 b	1.11 b	13.18 b	13.58	4.4 ab	0.75 ab
Hyb 4	5.10 b	5.14	1.47 b	1.17 a	13.31 b	13.94	5.6 a	1.0 a
PD								
PD 1	5.64	4.99	1.66	1.18	13.67 b	13.65	4.3	0.52
PD 2	5.75	5.22	1.65	1.17	14.04 a	14	5.2	0.75
Nr								
Nr 1	4.78 b	3.51 b	1.47 b	0.86 c	11.95 c	11.27 d	5.1	1.54 a
Nr 2	5.93 a	5.52 a	1.75 a	1.20 b	13.79 b	13.55 c	4.5	0.21 b
Nr 3	6.09 a	5.57 a	1.67 a	1.32 a	14.69 a	14.48 b	4.9	0.46 b
Nr 4	5.96 a	5.74 a	1.74 a	1.31 a	14.97 a	15.88 a	4.5	0.33 b
Anova								
Hyb	**	ns	**	*	**	ns	*	*
PD	ns	ns	ns	ns	*	ns	ns	ns
Nr	**	**	**	**	**	**	ns	**
Hyb x PD	**	ns	*	ns	ns	ns	*	ns
Hyb x Nr	*	ns	**	ns	ns	ns	ns	ns
PD x Nr	ns	ns	ns	ns	ns	ns	ns	ns
Hyb x PD x Nr	ns	ns	**	ns	ns	ns	ns	ns

ns = not significant; * = P < 0.05; ** = P < 0.01; *** = P < 0.0001

Table 2.13 - Grain yield (155 g kg⁻¹ moisture) from combine harvest (GY), grain harvest index (GHI), kernel number (KN), kernel weight (KW), at physiological maturity for all maize hybrids (Hybrid 1 = AQUAmax™ P1151, Hybrid 2 = P1162, Hybrid 3 = AQUAmax™ P1498, and Hybrid 4 = 33D49) grown at two plant densities (PD1=79,000, PD2=104,000 pl ha⁻¹) and four N rates (Nr1 = 0, Nr2 = 134, Nr 3 = 202, and Nr 4 = 269 kg ha⁻¹) in 2012

	GY+ (Mg ha ⁻¹)	KN (kernel m ⁻²)	KW (mg kernel ⁻¹)
Hybrid			
Hyb 1	6.81	1843 b	347 a
Hyb 2	6.94	2188 b	337 ab
Hyb 3	7.12	1819 b	352 a
Hyb 4	8.57	3101 a	323 b
PD			
PD 1	8.13 a	2154	346 a
PD 2	6.59 b	2322	334 b
Nr			
Nr 1	6.59 b	2163	321 b
Nr 2	7.56 a	2376	351 a
Nr 3	7.60 a	2067	347 a
Nr 4	7.67 a	2346	340 a
Anova			
Hyb	ns	**	**
PD	***	ns	*
Nr	**	ns	**
Hyb x PD	**	**	**
Hyb xNr	ns	ns	ns
PD x Nr	ns	ns	ns
Hyb x PD x Nr	ns	ns	ns

ns = not significant; *=P<0.05; **=P<0.01; ***=P<0.0001

+ = based in 5 reps

Table 2.14 - Grain yield (155 g kg⁻¹ moisture) from combine harvest (GY), grain harvest index (GHI), kernel number (KN), kernel weight (KW), at physiological maturity for all maize hybrids (Hybrid 1 = AQUAmax™ P1151, Hybrid 2 = P1162, Hybrid 3 = AQUAmax™ P1498, Hybrid 4 = 33D49, and Hybrid 5 = P1184) grown at two plant densities (PD1 = 78,000, PD2 = 99,000 pl ha⁻¹) and four N rates (Nr1 = 0, Nr2 = 134, Nr3 = 202, and Nr4 = 269 kg ha⁻¹) in 2013

	GY+ (Mg ha ⁻¹)	KN (kernel m ⁻²)	KW (mg kernel ⁻¹)
Hybrid			
Hyb 1	13.24 a	4074 c	295 a
Hyb 2	12.96 a	3827 d	304 a
Hyb 3	12.68 a	4522 b	249 b
Hyb 4	11.56 b	4839 a	227 c
PD			
PD 1	12.61	4196 b	274 a
PD 2	12.71	4435 a	263 b
Nr			
Nr 1	7.44 d	2773 c	247 c
Nr 2	13.38 c	4672 b	265 b
Nr 3	14.46 b	4898 ab	278 a
Nr 4	15.14 a	4919 a	286 a
Anova			
Hyb	**	**	**
PD	ns	**	**
Nr	**	**	**
Hyb x PD	ns	ns	ns
Hyb x Nr	ns	ns	ns
PD x Nr	ns	ns	ns
Hyb x PD x Nr	ns	ns	ns

ns = not significant; * = P < 0.05; ** = P < 0.01; *** = P < 0.0001.

+ = based in 5 reps

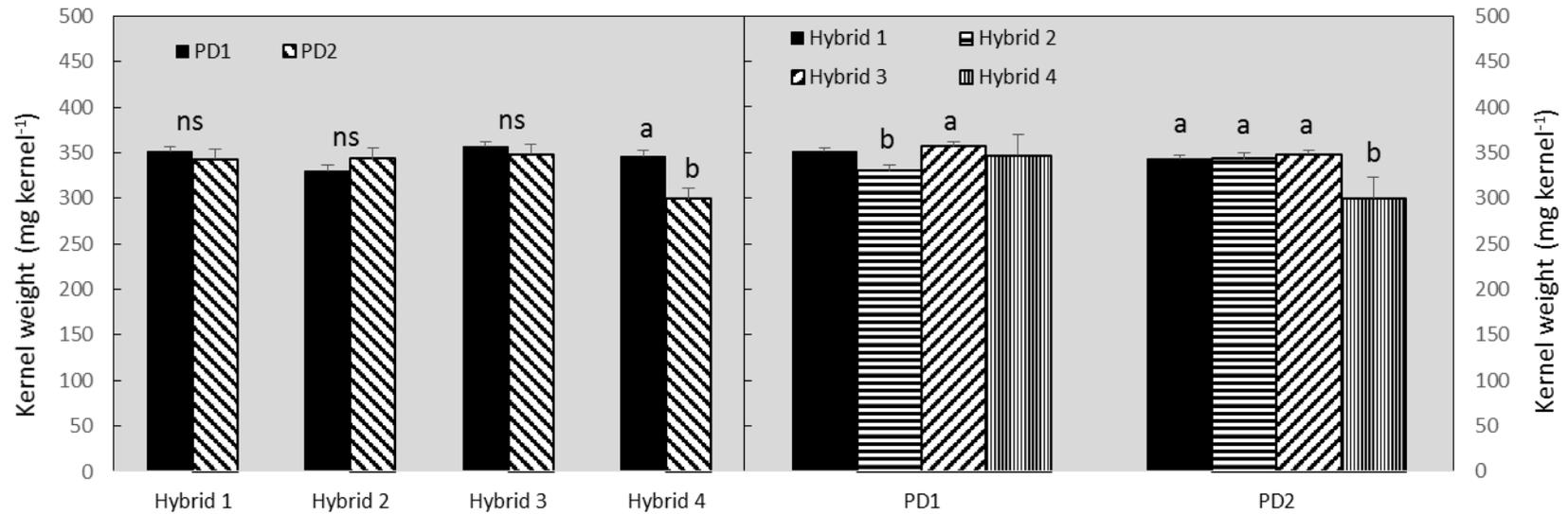


Figure 2.9 - Mean separation test for kernel weight (mg kernel⁻¹) in relation to the factorial Hybrid x PD, for each hybrid (Hybrid 1 = AQUAmax™ P1151, Hybrid 2 = P1162, Hybrid 3 = AQUAmax™ P1498, Hybrid 4 = 33D49) and both plant densities (PD1 = 79,000; and PD2 = 104,000 pl ha⁻¹), when averaged across all 4 N rates in 2012

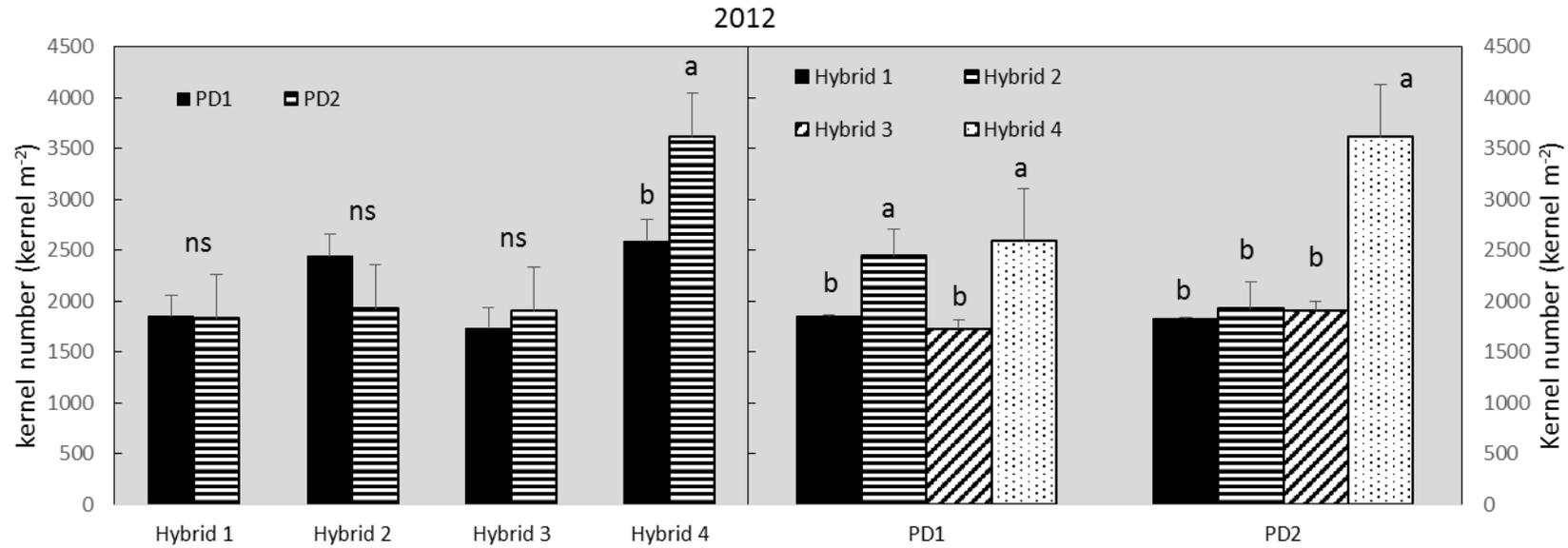


Figure 2.10 - Mean separation test for kernel number (ear⁻¹) in relation to the factorial Hybrid x PD, for each hybrid (Hybrid 1 = AQUAmaxTM P1151, Hybrid 2 = P1162, Hybrid 3 = AQUAmaxTM P1498, Hybrid 4 = 33D49) and both plant densities (PD1 = 79,000; and PD2 = 104,000 pl ha⁻¹), when averaged across all 4 N rates in 2012

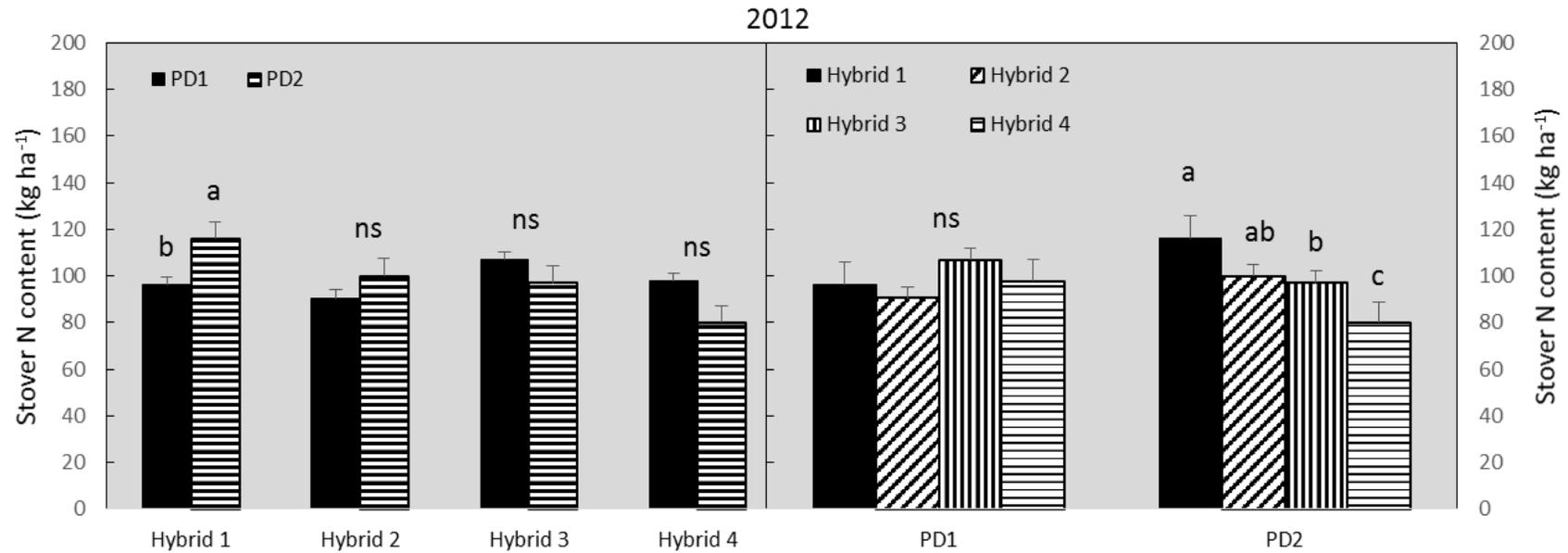


Figure 2.11 - Mean separation test for stover N content (kg ha⁻¹) in relation to the factorial Hybrid x PD, for each hybrid (Hybrid 1 = AQUAmaxTM P1151, Hybrid 2 = P1162, Hybrid 3 = AQUAmaxTM P1498, Hybrid 4 = 33D49) and both plant densities (PD1 = 79,000; and PD2 = 104,000 pl ha⁻¹), when averaged across all 4 N rates in 2012

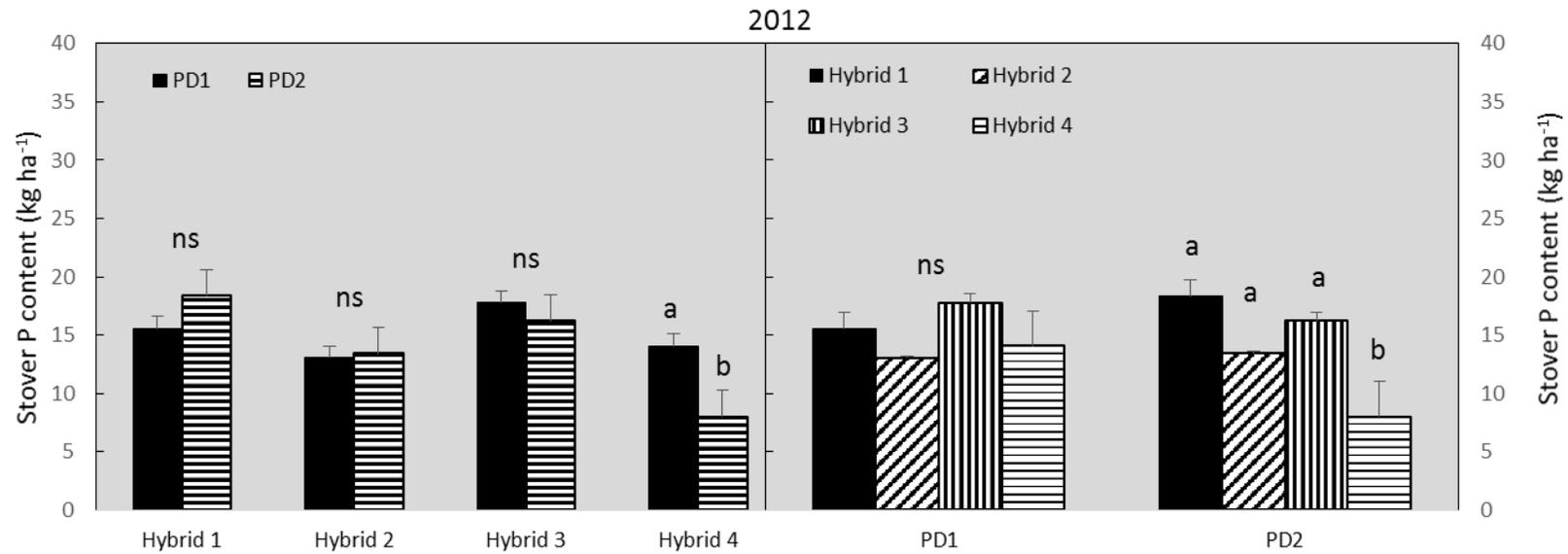


Figure 2.12 - Mean separation test for stover P content (kg ha⁻¹) in relation to the factorial Hybrid x PD, for each hybrid (Hybrid 1 = AQUAmax™ P1151, Hybrid 2 = P1162, Hybrid 3 = AQUAmax™ P1498, Hybrid 4 = 33D49) and both plant densities (PD1 = 79,000; and PD2 = 104,000 pl ha⁻¹), when averaged across all N rates in 2012

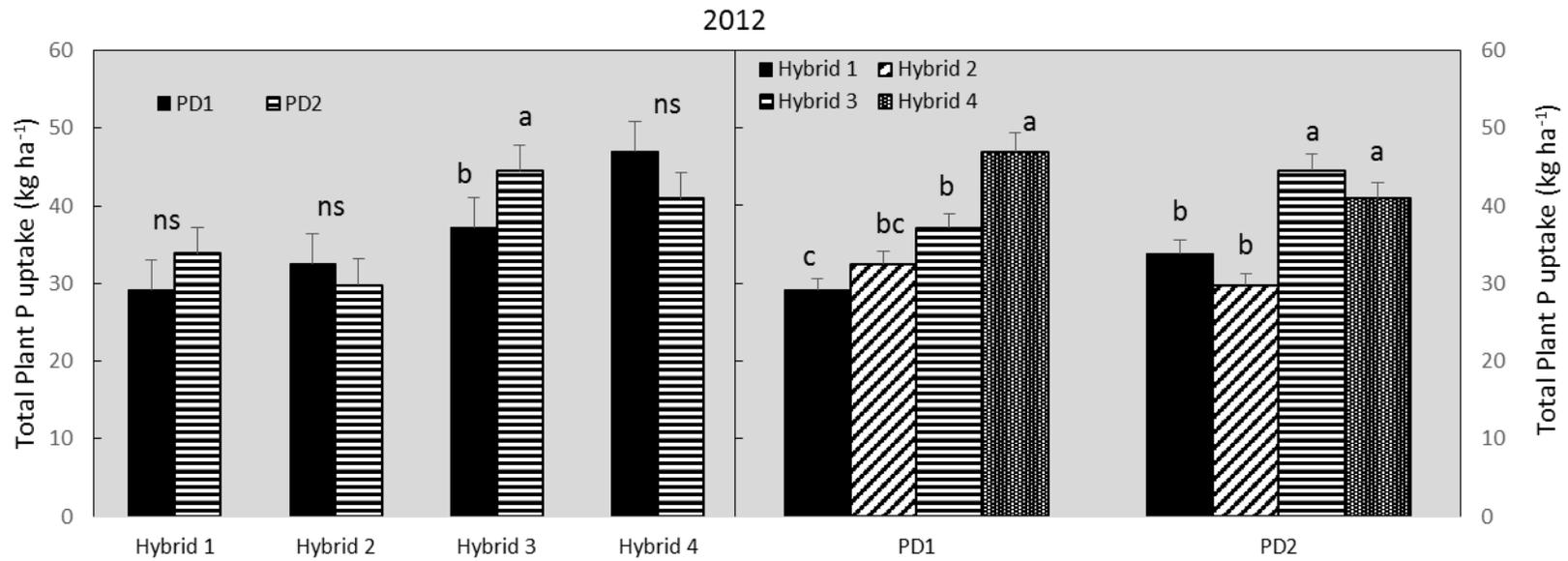


Figure 2.13 - Mean separation test for total plant P uptake (kg ha⁻¹) in relation to the factorial Hybrid x PD, for each hybrid (Hybrid 1 = AQUAmaxTM P1151, Hybrid 2 = P1162, Hybrid 3 = AQUAmaxTM P1498, Hybrid 4 = 33D49) and both plant densities (PD1 = 79,000; and PD2 = 104,000 pl ha⁻¹), when averaged across all 4 N rates in 2012

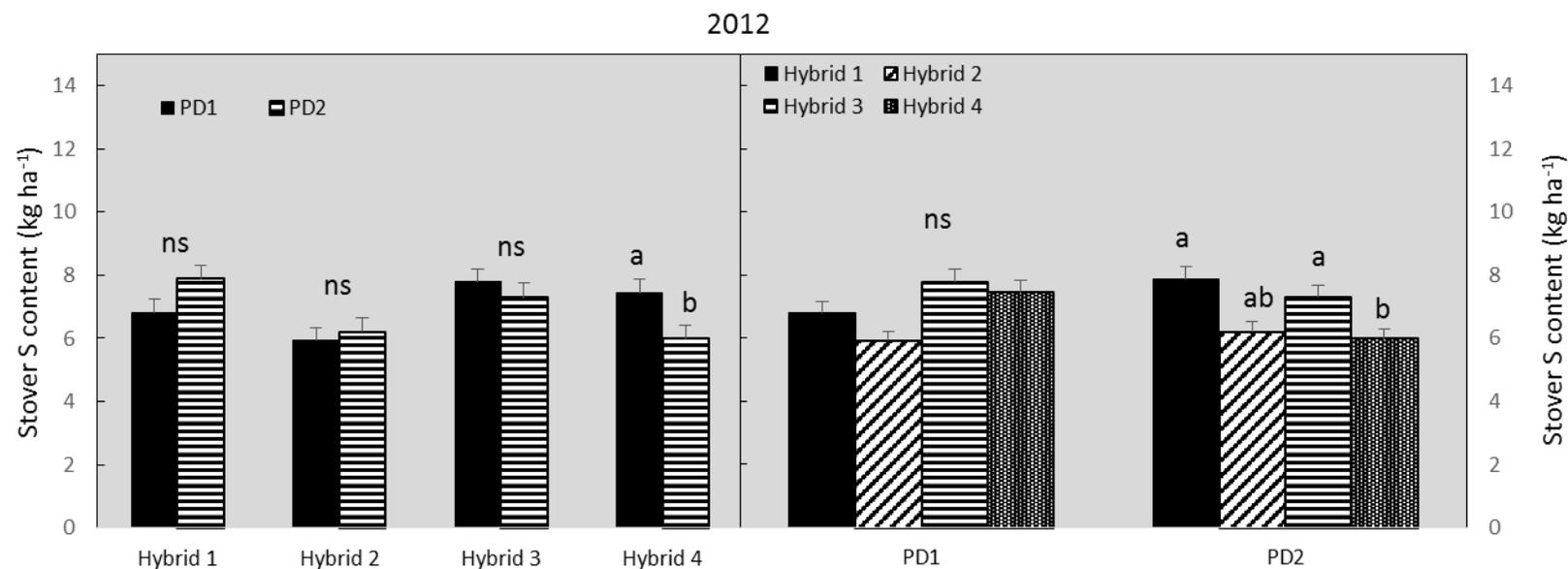


Figure 2. 14 - Mean separation test for stover S content (kg ha⁻¹) in relation to the factorial Hybrid x PD, for each hybrid (Hybrid 1 = AQUAmaxTM P1151, Hybrid 2 = P1162, Hybrid 3 = AQUAmaxTM P1498, Hybrid 4 = 33D49) and both plant densities (PD1 = 79,000; and PD2 = 104,000 pl ha⁻¹), when averaged across all 4 N rates in 2012

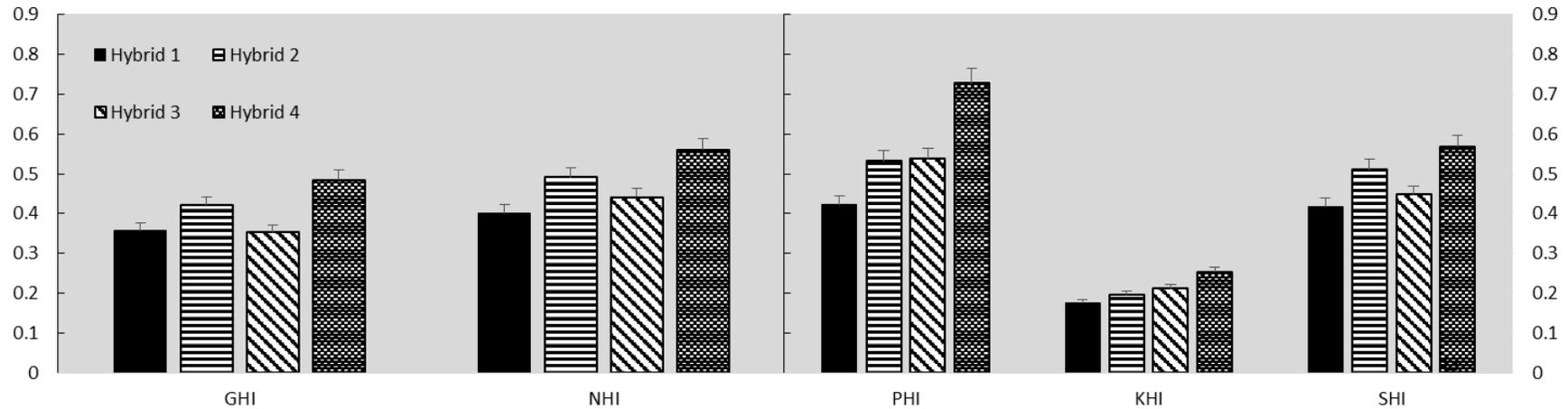


Figure 2.15 - Grain harvest index (GHI), nitrogen harvest index (NHI), phosphorus harvest index (PHI), potassium harvest index (KHI), and Sulfur harvest index (SHI) for each hybrid (Hybrid 1 = AQUAmax™ P1151, Hybrid 2 = P1162, Hybrid 3 = AQUAmax™ P1498, Hybrid 4 = 33D49) averaged across both plant densities (PD1 = 79,000; and PD2 = 104,000 pl ha⁻¹) and four N rate levels (Nr1 = 0, Nr2 = 134, Nr3 = 202, and Nr4 = 269 kg N sidedress ha⁻¹) in 2012

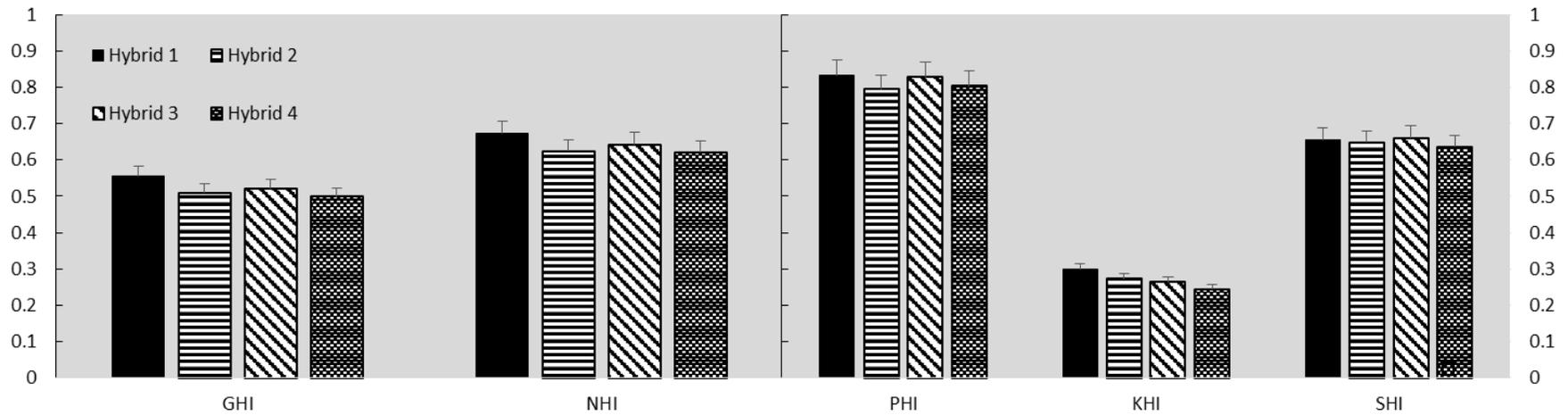


Figure 2.16 - Grain harvest index (GHI), nitrogen harvest index (NHI), phosphorus harvest index (PHI), potassium harvest index (KHI), and sulfur harvest index (SHI) for each hybrid (Hybrid 1 = AQUAmax™ P1151, Hybrid 2 = P1162, Hybrid 3 = AQUAmax™ P1498, Hybrid 4 = 33D49) averaged across both plant densities (PD1 = 78,000; and PD2 = 99,000 pl ha⁻¹) and each N rate level (Nr1 = 0, Nr2 = 134, Nr3 = 202, and Nr4 = 269 kg N sidedress ha⁻¹) in 2013

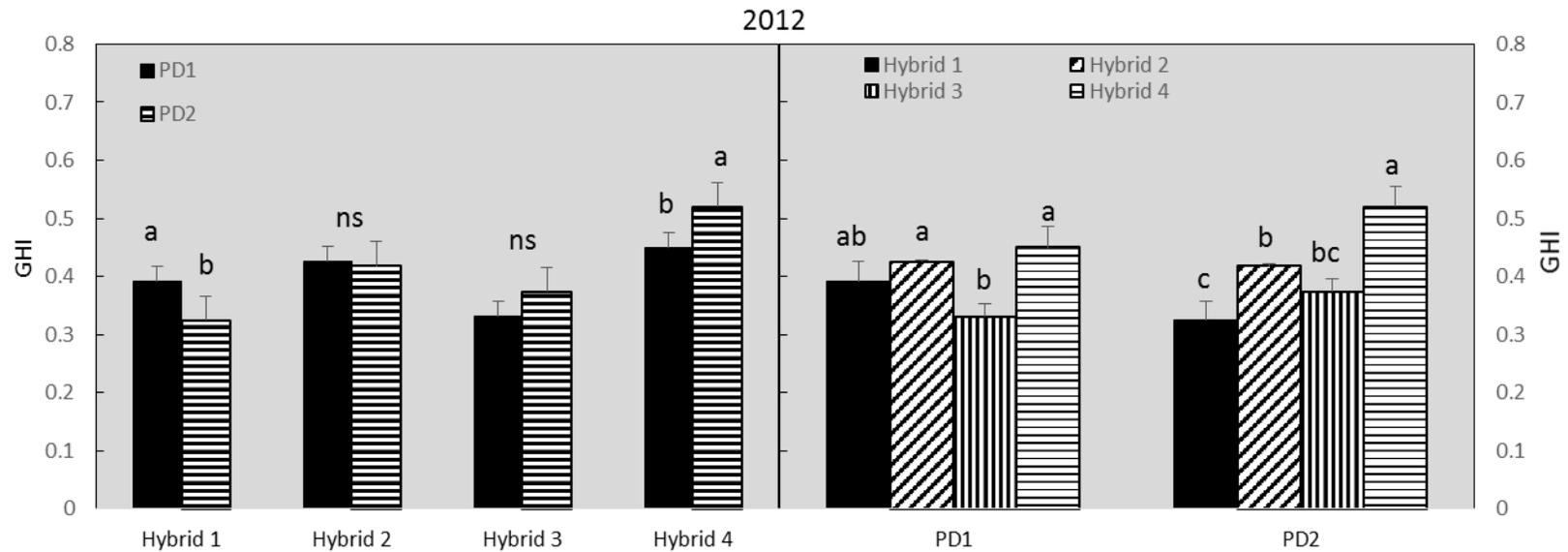


Figure 2.17 - Mean separation test for GHI (Grain Harvest Index) in relation to the factorial Hybrid x PD, for each hybrid (Hybrid 1 = AQUAmax™ P1151, Hybrid 2 = P1162, Hybrid 3 = AQUAmax™ P1498, Hybrid 4 = 33D49) and both plant densities (PD1 = 79,000; and PD2 = 104,000 pl ha⁻¹), when averaged across all N rates in 2012

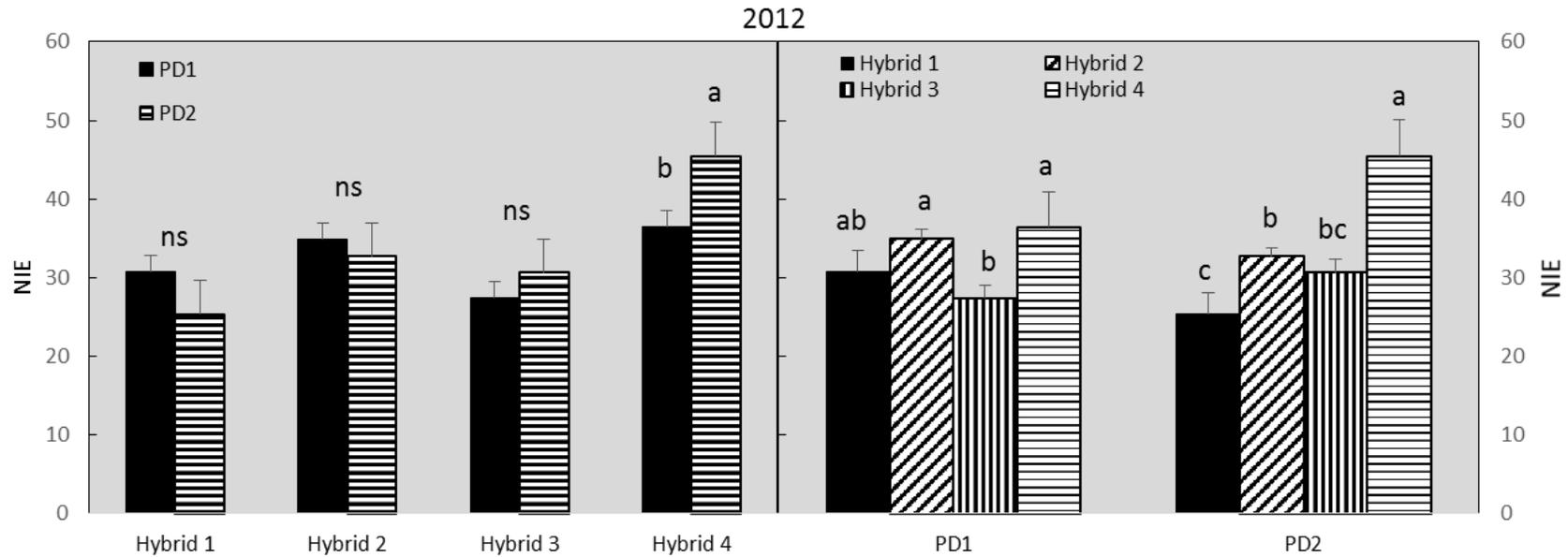


Figure 2.18 - Mean separation test for NIE (Nitrogen Internal Efficiency) in relation to the factorial Hybrid x PD, for each hybrid (Hybrid 1 = AQUAmax™ P1151, Hybrid 2 = P1162, Hybrid 3 = AQUAmax™ P1498, Hybrid 4 = 33D49) and both plant densities (PD1 = 79,000; and PD2 = 104,000 pl ha⁻¹), when averaged across all 4 N rates in 2012

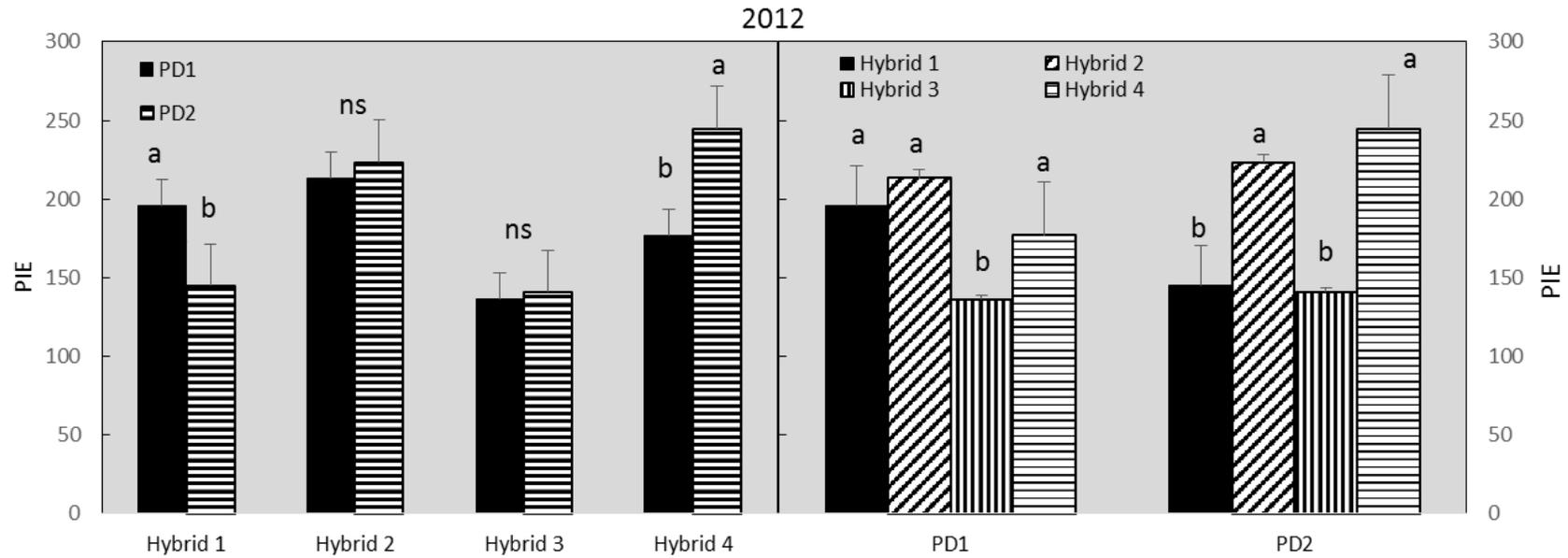


Figure 2.19 - Mean separation test for PIE (Phosphorus Internal Efficiency) in relation to the factorial Hybrid x PD, for each hybrid (Hybrid 1 = AQUAmax™ P1151, Hybrid 2 = P1162, Hybrid 3 = AQUAmax™ P1498, Hybrid 4 = 33D49) and both plant densities (PD1 = 79,000; and PD2 = 104,000 pl ha⁻¹), when averaged in all 4 N rates in 2012

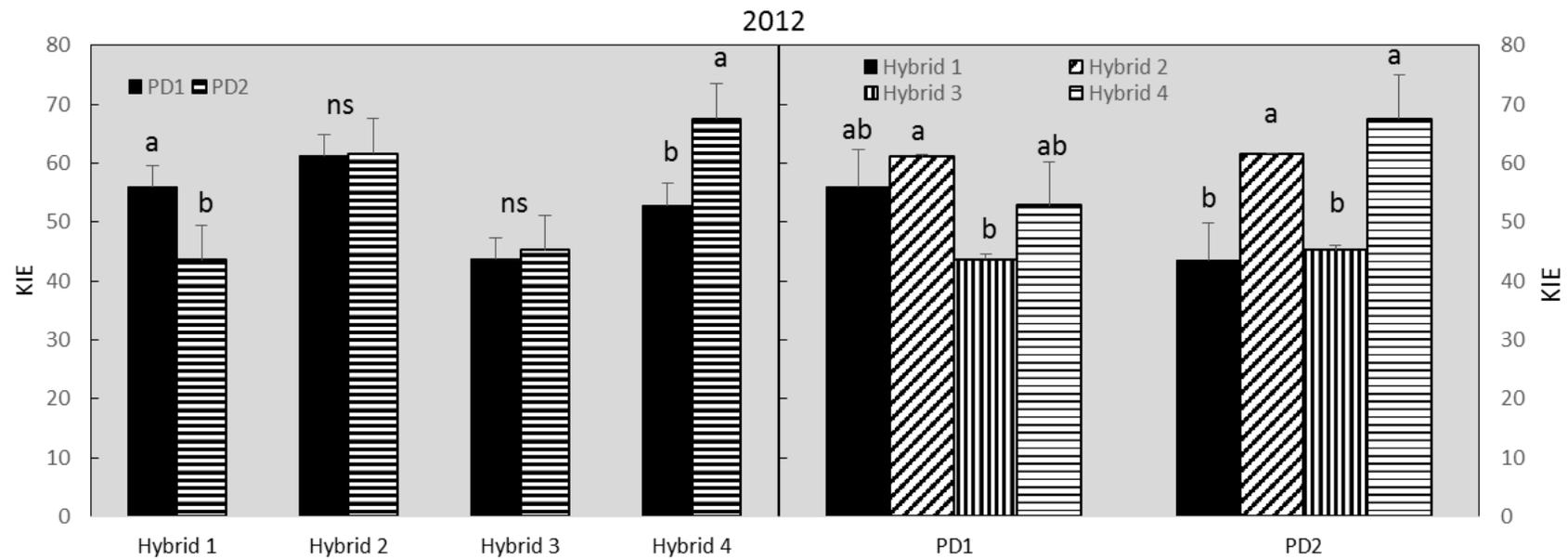


Figure 2.20 - Mean separation test for KIE (Potassium Internal Efficiency) in relation to the factorial Hybrid x PD, for each hybrid (Hybrid 1 = AQUAmax™ P1151, Hybrid 2 = P1162, Hybrid 3 = AQUAmax™ P1498, Hybrid 4 = 33D49) and both plant densities (PD1 = 79,000; and PD2 = 104,000 pl ha⁻¹), when averaged across all 4 n rates in 2012

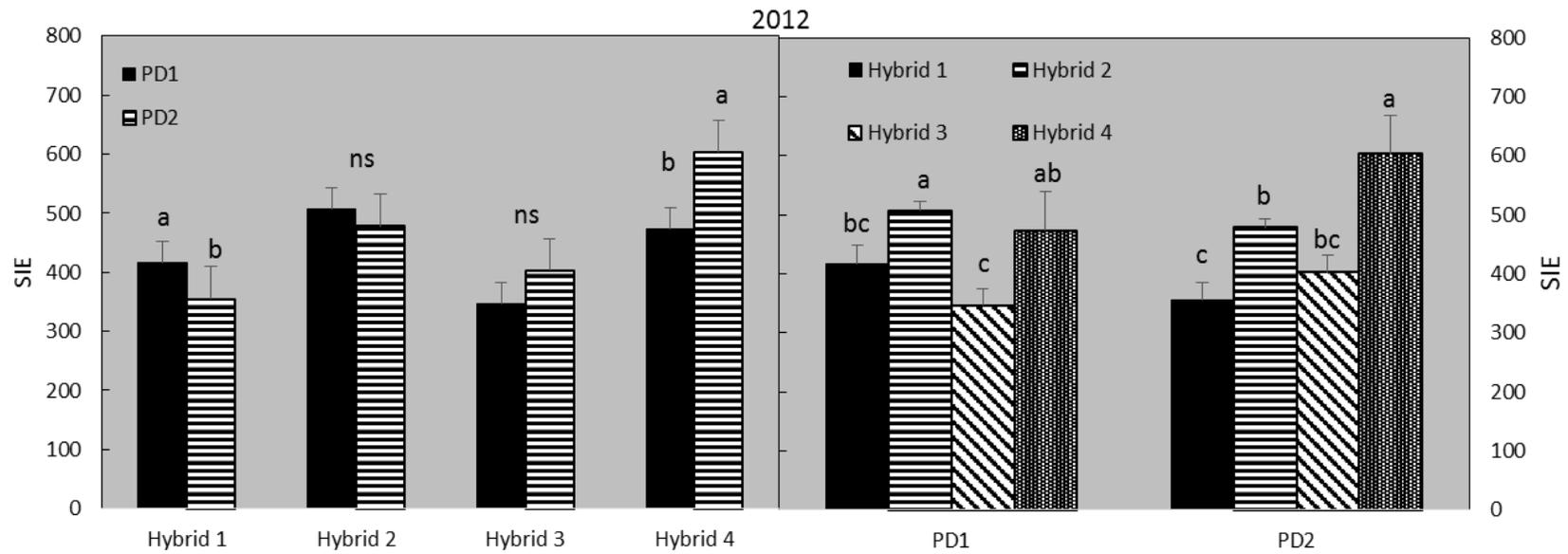


Figure 2.21 - Mean separation test for SIE (Sulfur Internal Efficiency) for the factorial Hybrid x PD, for all four hybrids (Hybrid 1 = AQUAmax™ P1151, Hybrid 2 = P1162, Hybrid 3 = AQUAmax™ P1498, Hybrid 4 = 33D49), and each plant density level (PD1 = 79,000; and PD2 = 104,000 pl ha⁻¹), when averaged in all 4 N rates in 2012

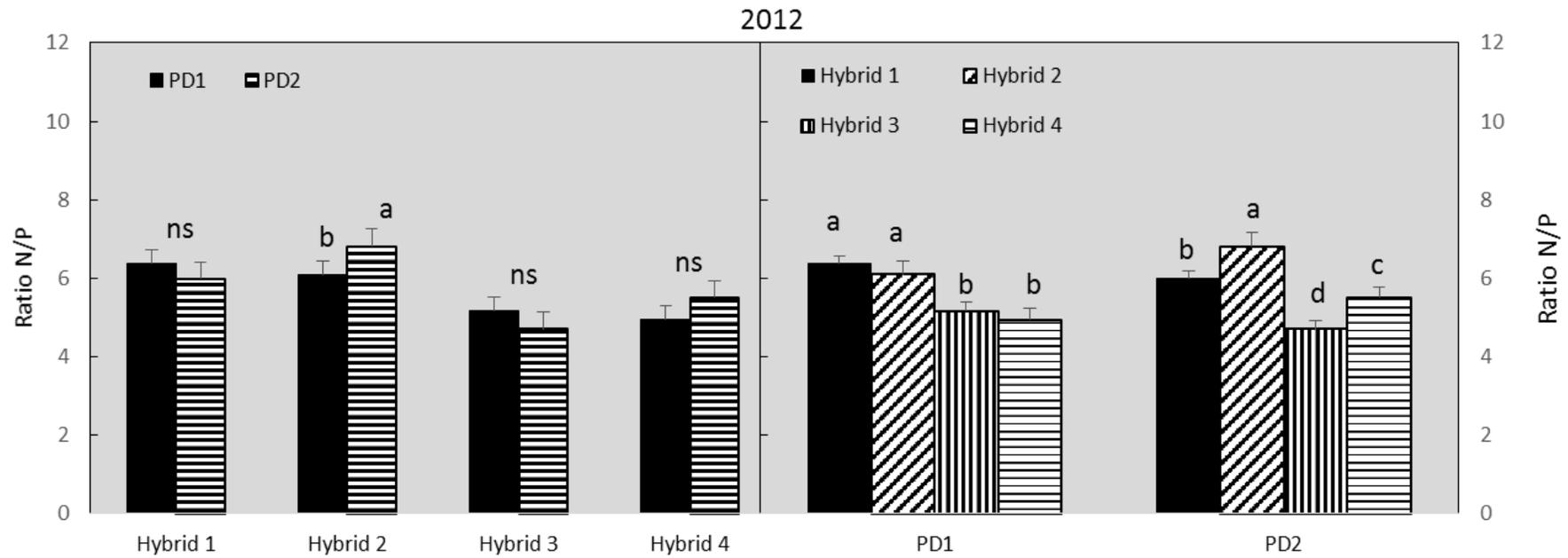


Figure 2.22 - Mean separation test for Ratio N/P in relation to the factorial Hybrid x PD, for each hybrid (Hybrid 1 = AQUAmax™ P1151, Hybrid 2 = P1162, Hybrid 3 = AQUAmax™ P1498, Hybrid 4 = 33D49) averaged across both plant densities (PD1 = 79,000; and PD2 = 104,000 pl ha⁻¹), when averaged across all 4 N rates in 2012

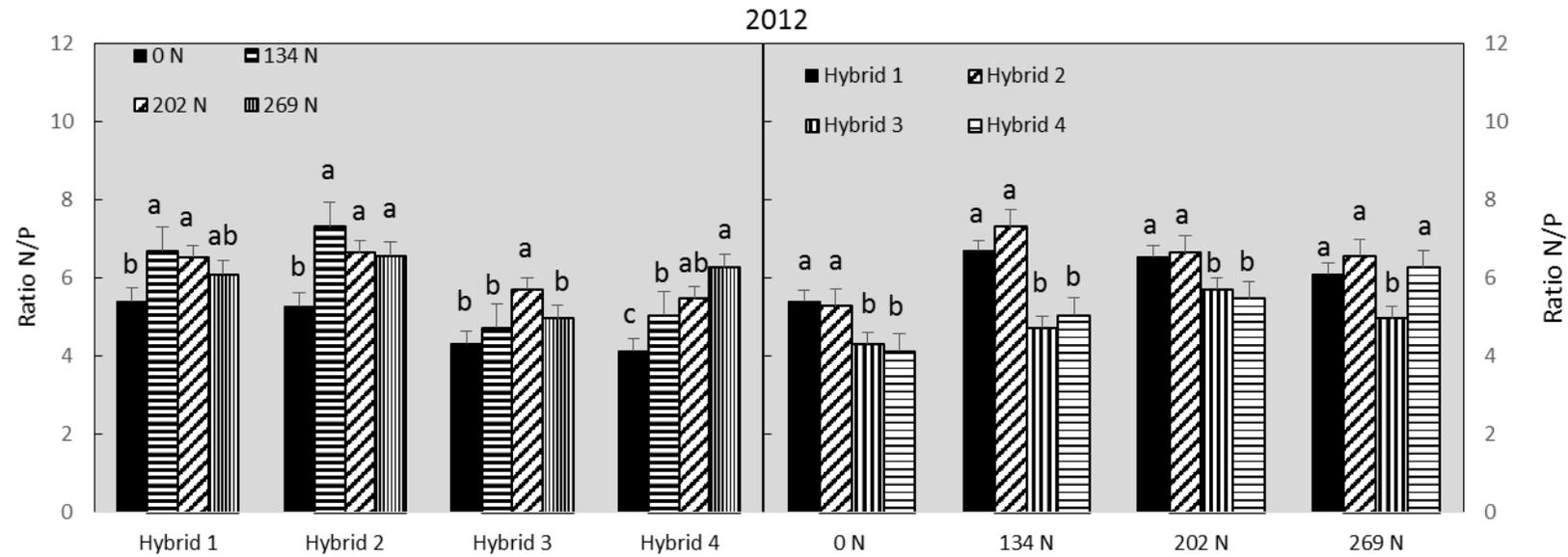


Figure 2.23 - Mean separation test for Ratio N/P in relation to the factorial Hybrid x N rate, for each hybrid (Hybrid 1 = AQUAmax™ P1151, Hybrid 2 = P1162, Hybrid 3 = AQUAmax™ P1498, Hybrid 4 = 33D49) and four N rate levels (Nr1 = 0, Nr2 = 134, Nr3 = 202, and Nr4 = 269 kg N sidedress ha⁻¹) when averaged across both plant densities in 2012

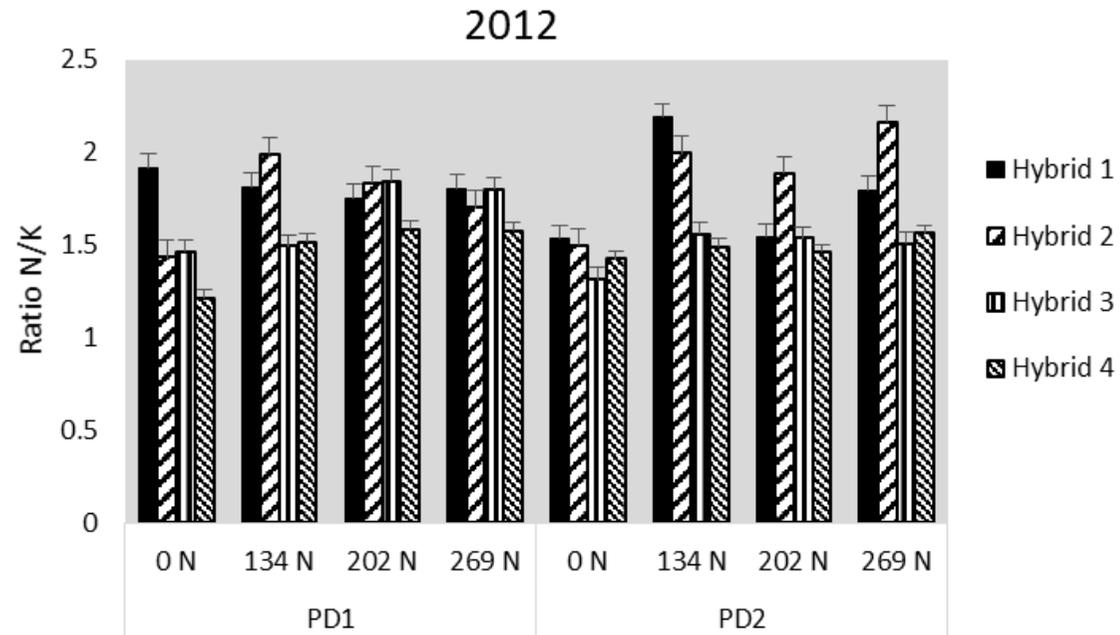


Figure 2.24 - Ratio N/P for the factorial Hybrid x PD x N rate at physiological maturity for all four hybrids (Hybrid 1 = AQUAmax™ P1151, Hybrid 2 = P1162, Hybrid 3 = AQUAmax™ P1498, Hybrid 4 = 33D49), each plant density level (PD1 = 79,000; and PD2 = 104,000 pl ha⁻¹), and all four N rate levels (Nr1 = 0, Nr2 = 134, Nr3 = 202, and Nr4 = 269 kg N sidedress ha⁻¹), for 2012

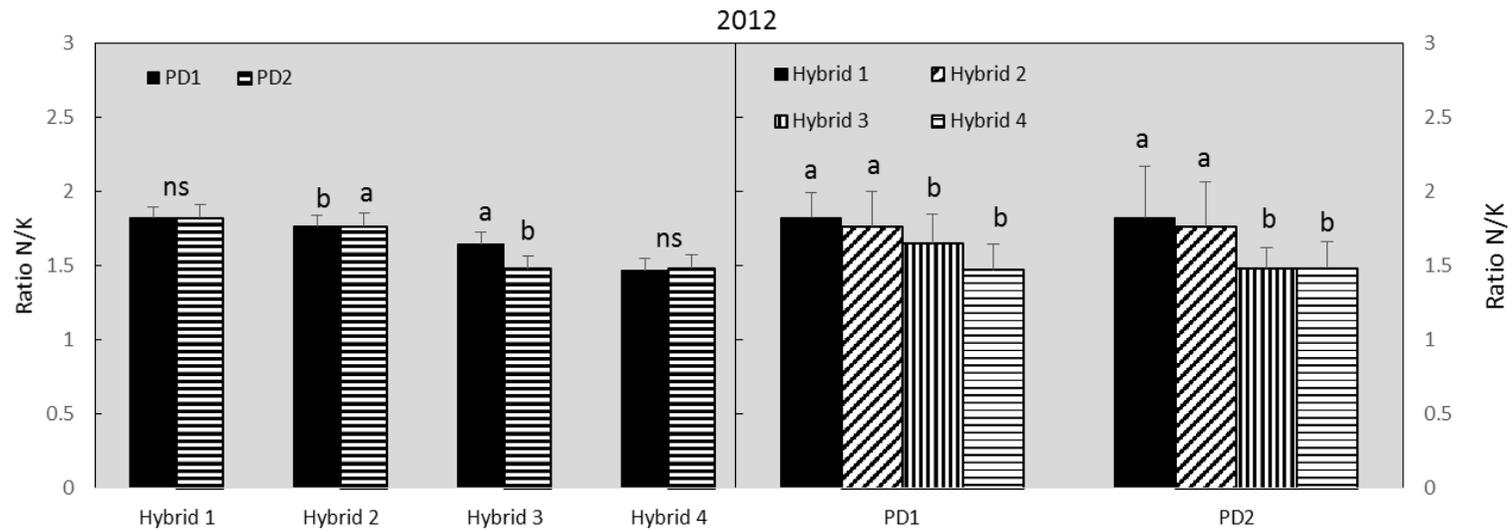


Figure 2.25 - Mean separation test for Ratio N/P for the factorial Hybrid x PD, for all four hybrids (Hybrid 1 = AQUAmax™ P1151, Hybrid 2 = P1162, Hybrid 3 = AQUAmax™ P1498, Hybrid 4 = 33D49), and each plant density level (PD1 = 79,000; and PD2 = 104,000 pl ha⁻¹), when averaged across all N rates in 2012

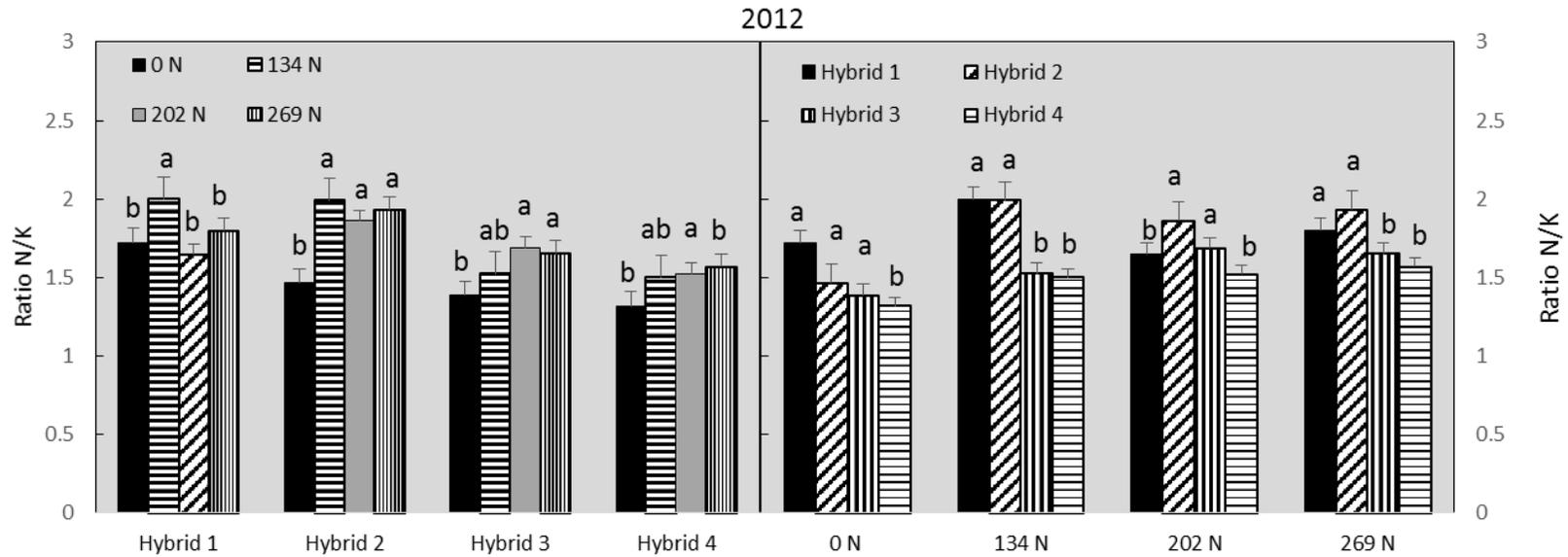


Figure 2.26 - Mean separation test for Ratio N/K for the factorial Hybrid x N rate, for all four hybrids (Hybrid 1 = AQUAmax™ P1151, Hybrid 2 = P1162, Hybrid 3 = AQUAmax™ P1498, Hybrid 4 = 33D49), and all four N rate levels (Nr1 = 0, Nr2 = 134, Nr3 = 202, and Nr4 = 269 kg N sidedress ha⁻¹), when averaged across both plant density levels in 2012

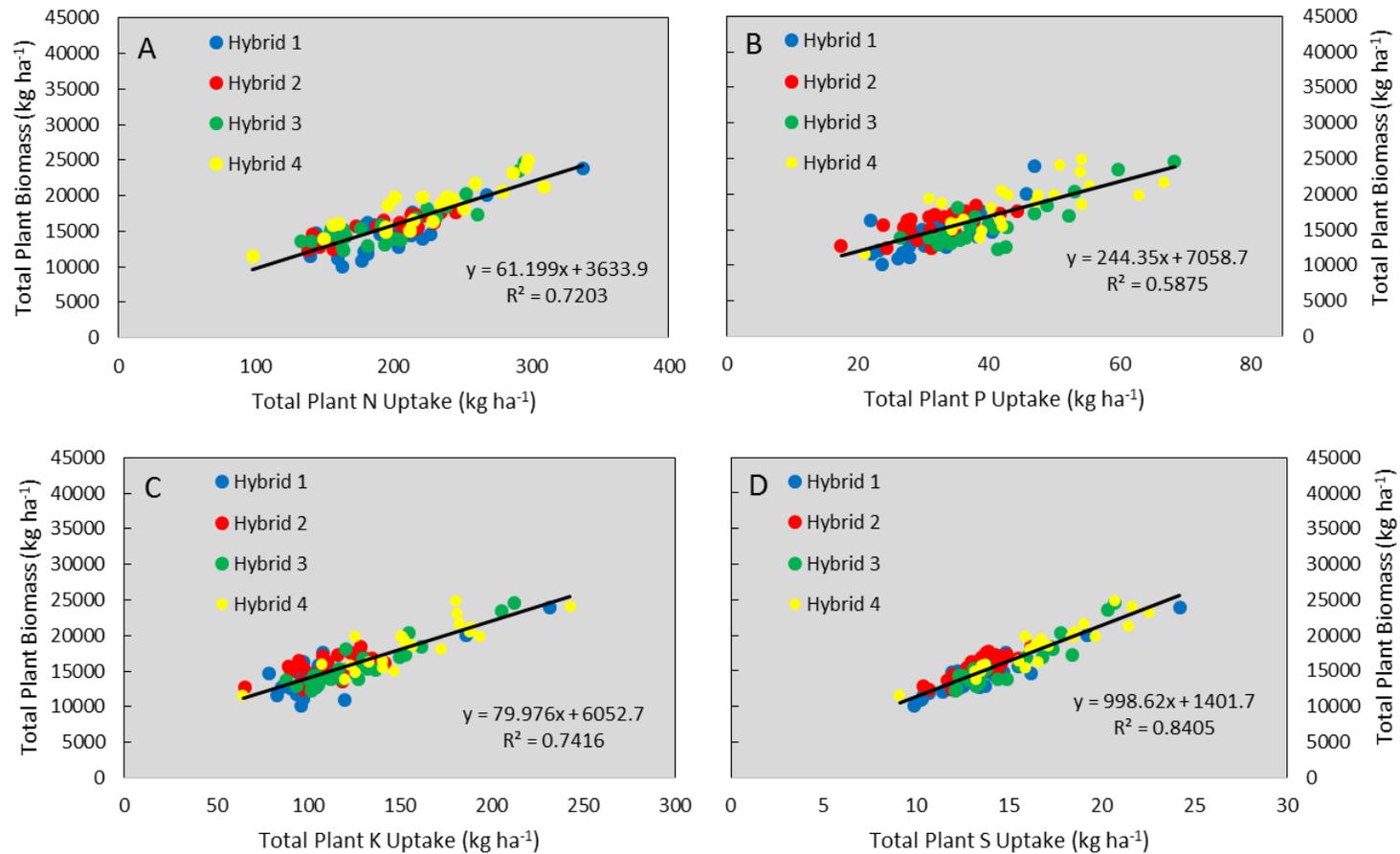


Figure 2.27 - Total plant nutrient uptake for Nitrogen (A), Phosphorus (B), Potassium (C), and Sulfur (D) relative to the total plant biomass at physiological maturity for all four hybrids (Hybrid 1 = AQUAmaxTM P1151, Hybrid 2 = P1162, Hybrid 3 = AQUAmaxTM P1498, Hybrid 4 = 33D49), each plant density level (PD1 = 79,000; and PD2 = 104,000 pl ha⁻¹), and all four N rate levels (Nr1 = 0, Nr2 = 134, Nr3 = 202, and Nr4 = 269 kg N sidedress ha⁻¹) in 2012

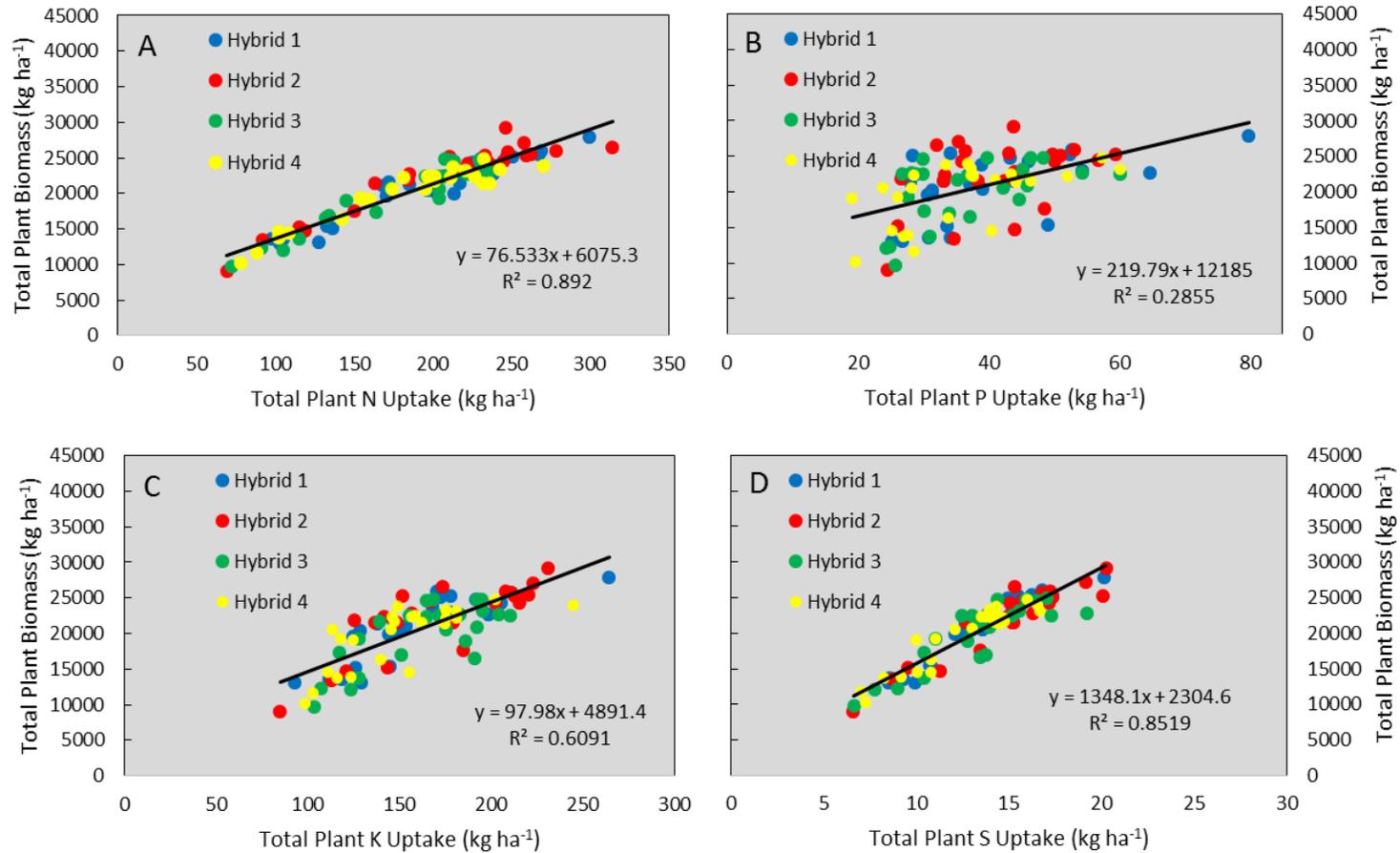


Figure 2.28 - Total plant nutrient uptake for Nitrogen (A), Phosphorus (B), Potassium (C), and Sulfur (D) relative to the total plant biomass at physiological maturity for all four hybrids (Hybrid 1 = AQUAmaxTM P1151, Hybrid 2 = P1162, Hybrid 3 = AQUAmaxTM P1498, Hybrid 4 = 33D49), each plant density level (PD1 = 78,000; and PD2 = 99,000 pl ha⁻¹), and all four N rate levels (Nr1 = 0, Nr2 = 134, Nr3 = 202, and Nr4 = 269 kg N sidedress ha⁻¹) in 2013

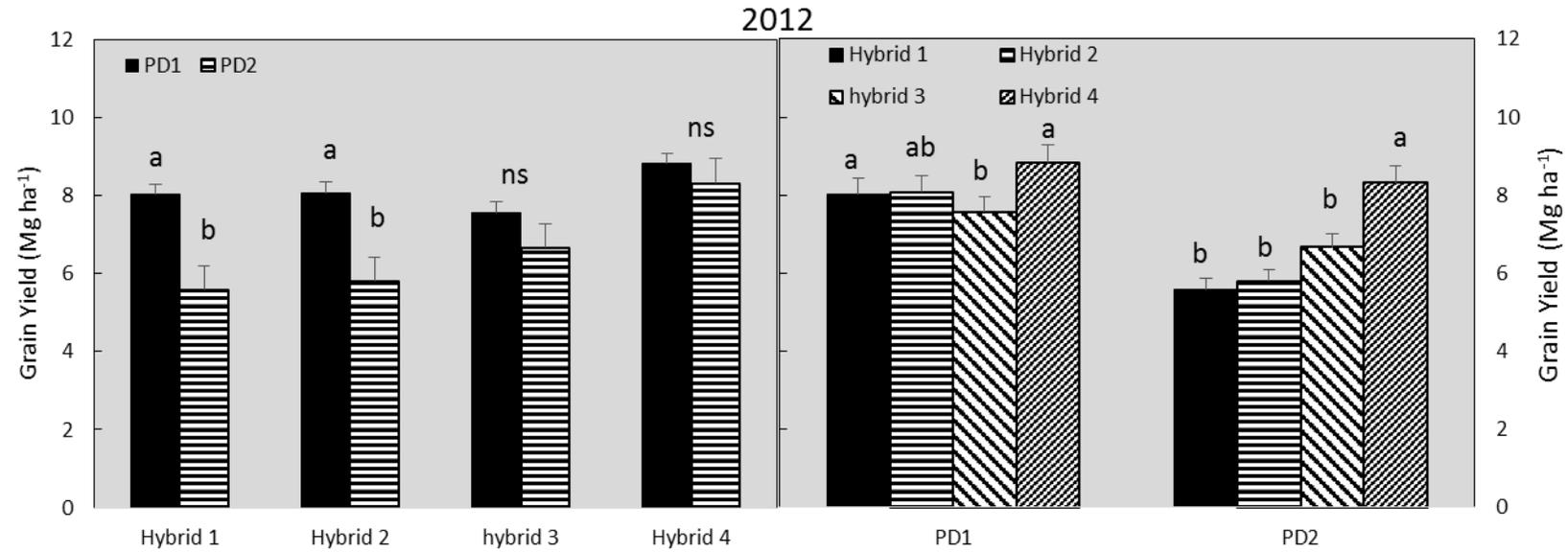


Figure 2.29 - Mean separation test for grain yield for the factorial Hybrid x PD, for all four hybrids (Hybrid 1 = AQUAmax™ P1151, Hybrid 2 = P1162, Hybrid 3 = AQUAmax™ P1498, Hybrid 4 = 33D49), and each plant density level (PD1 = 79,000; and PD2 = 104,000 pl ha⁻¹), when averaged across all 4 N rates in 2012

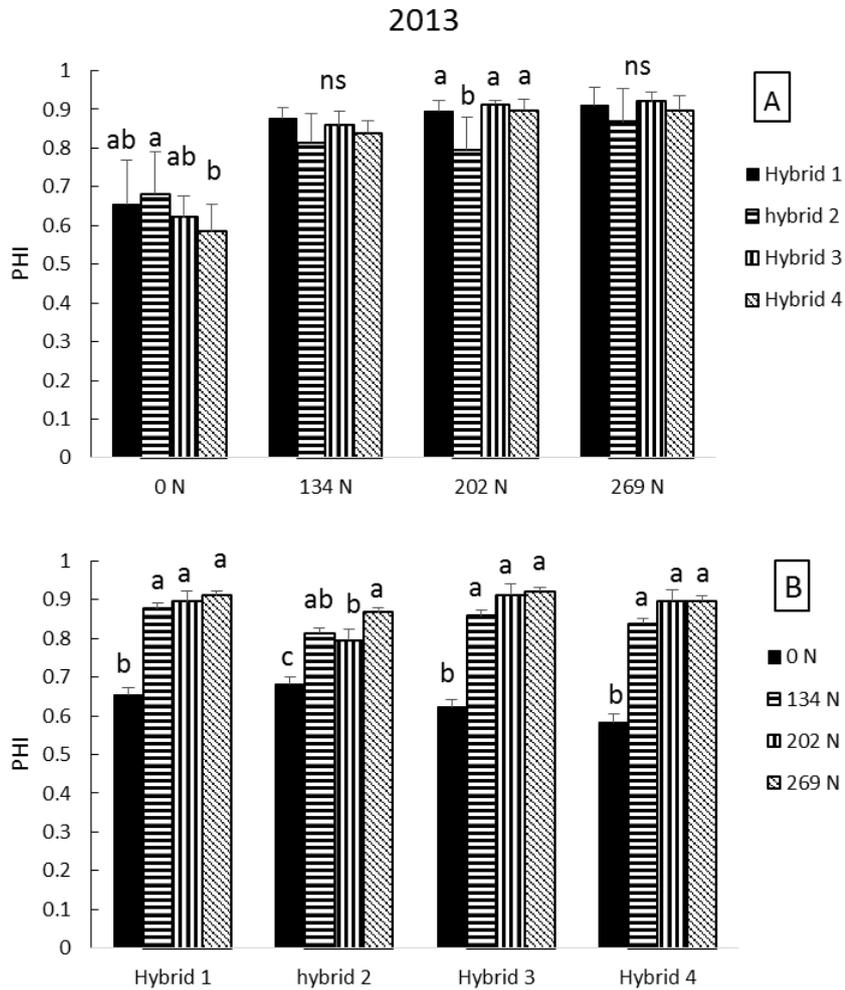


Figure 2.30 - Mean separation test for PHI (phosphorus harvest index), in A and B, for the factorial Hybrid x N rate, for all four hybrids (Hybrid 1 = AQUAmax™ P1151, Hybrid 2 = P1162, Hybrid 3 = AQUAmax™ P1498, Hybrid 4 = 33D49), and all four N rate levels (Nr1 = 0, Nr2 = 134, Nr3 = 202, and Nr4 = 269 kg N sidedress ha⁻¹), when averaged across both plant density in 2012

References

ABENDROTH, L.J.; ELMORE R.W.; BOYER M.J., MARLAY, S.K. **Corn growth and development**. Ames: Iowa State University Extension, 2011. 50 p. PMR 1009.

AGRIANUAL. **Anuário da agricultura brasileira**. São Paulo: FNP, 2009. p. 371-376.

AKINTOYE, H.A.; KLING, J.G.; LUCAS, E.O. N-use efficiency of single, double and synthetic maize lines grown at four N levels in three ecological zones of West Africa. **Field Crops Research** Amsterdam, v. 60, n. 3, p. 189-199, 1999.

ALMEIDA, M.L.; SANGOI, L. Aumento da densidade de plantas de milho para regiões de curta estação estival de crescimento. **Pesquisa Agropecuária Gaúcha**, Porto Alegre, v. 2, n. 2, p. 179-183, 1996.

ALVES, A.C.; OLIVEIRA, P.P.A.; HERLING, V.R.; TRIVELIN P.C.O.; CERQUEIRA-LUZ, P.H.; ALVES, T.C.; ROCHETTI, R.C.; BARIONI JÚNIOR, W. New methods to quantify NH₃ volatilization from fertilized surface soil with urea. **Revista Brasileira de Ciência do Solo**, Viçosa, v. 35, n. 1, p. 133-140, 2011.

AMADO, T.J.C.; MIELNICZUK, J.; AITA, C. Recomendação de adubação nitrogenada para o milho no RS e SC adaptada ao uso de culturas de cobertura do solo, sob sistema plantio direto. **Revista Brasileira de Ciência do Solo**, Viçosa, v. 26, p. 241-248, 2002.

ANDRADE, F.H. Analysis of growth and yield of maize, sunflower and soybean grown at Balcarce, Argentina. **Field Crops Research**, Amsterdam, v. 41, n. 1, p. 1-12, 1995.

ANDRADE, F.H.; VEGA, C.; UHART, S.O. Kernel number determination in maize. **Crop Science**, Madison, v. 39, n. 2, p. 453-459, 1999.

ANGHINONI, I.; VOLKART, K.; FATTORE, C.; ERNANI, P.R. Morfologia de raízes e cinética da absorção de nutrientes em diversas espécies e genótipos de plantas. **Revista Brasileira de Ciência do Solo**, Viçosa, v. 13, n. 1, p. 355-361, 1989.

APHALO, P.J.; BALLAR, C.L.; SCOPEL, A.L. Plant-plant signaling, the shade-avoidance response and competition. **Journal of Experimental Botany**, Oxford, v. 50, p. 1629-1634, 1999.

ARGENTA, G.S.; SILVA, P.R.F.; BORTOLINI, C.G.; FORSTHOFER, E.L.; MANJABOSCO, E.A.; BEHEREGARAY NETO, V. Resposta de híbridos simples à redução do espaçamento entre linhas. **Pesquisa Agropecuária Brasileira**, Brasília, v. 36, n. 1, p. 71-78, 2001.

ARGENTA, G.; SILVA, P.R.F.; BORTOLINI, C.G.; FORSTHOFER, E.L.; STRIEDER, M.L.; STEFANI, G.F. Relação entre teor de clorofila extraível e leitura do clorofilômetro na folha de milho. In: CONGRESSO NACIONAL DE MILHO E SORGO, 23., 2000, Uberlândia. **Resumos...** Uberlândia: ABMS, 2000. p. 197.

BALIGAR, V.C.; BARBER, S.A. Genotypic differences of corn for ion uptake. **Agronomy Journal**, Madison, v. 71, n. 5, p. 870-873, 1979.

BÄNZINGER, M.; EDMEADES, G.O.; BECK, D.; BELLON, M.R. **Breeding for drought and nitrogen stress tolerance in maize: from theory to practice**. Mexico: CIMMYT, 2000. 68 p.

BATES, T.R.; LYNCH, J.P. Stimulation of root hair elongation in *Arabidopsis thaliana* by low phosphorus availability. **Plant Cell Environ**, Chichester, v. 19, p. 529-538, 1996.

BECKER, J.; BEAN, B.; XUE, Q.; MAREK, T. **Pioneer Optimum AquaMax™ first generation drought tolerant corn trial: progress report**. Texas A&M Agrilife Research, 2012. Disponível em: <<http://amarillo.tamu.edu/files/2010/11/2011-AQUAmax-trial.pdf>>. Acesso em: 23 jan. 2015.

BELOW, F.E.; CHRISTENSEN, L.E.; REED, A.J.; HAGEMAN, R.H. Availability of reduced N and carbohydrates for ear development of maize. **Plant Physiology**, Lancaster, v. 68, n. 5, p. 1186-1190, 1981.

BERNARDI, A.C.C.; MACHADO, P.L.O.A.; FREITAS, P.L.; COELHO, M.R.; LEANDRO, W.M.; OLIVEIRA JÚNIOR, J.P.; OLIVEIRA, R. P.; SANTOS, H.G.; MADARI, B.E.; CARVALHO, M.C.S. **Correção do solo e adubação no sistema de plantio direto nos cerrados**. Rio de Janeiro: Embrapa Solos, 2003. 22 p.

BOOMSMA, C.R.; VYN, T.J. Maize drought tolerance: Potential improvements through arbuscular mycorrhizal symbiosis? **Field Crops Research**, Amsterdam, v. 108, n. 1, p. 14-31, 2008.

BORCH, K.; BOUMA, T.J.; LYNCH, J.P.; BROWN, K.M. Ethylene: a regulator of root architectural responses to soil phosphorus availability. **Plant Cell Environ**, Chichester, v. 22, p. 425-431, 1999.

BORRÁS, L.; WESTGATE, M.E. Predicting maize kernel sink capacity early in development. **Field Crops Research**, Amsterdam, v. 95, p. 223-233, 2006.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. **Cadeia produtiva do milho**. Brasília: IICA/MAPA/SPA, 2007. 108 p.

_____. **Milho**. Disponível em: <<http://www.agricultura.gov.br/vegetal/culturas/milho>>. Acesso em: 06 ago. 2014.

BURZACO, J.P.; CIAMPITTI, I.A.; VYN, T.L. Nitrapyrin impacts on maize yield and nitrogen use efficiency with spring-applied nitrogen: field studies vs. meta-analysis comparison. **Agronomy Journal**, Madison, v. 106, n. 2, p. 753-760. 2014.

ÇAKIR, R. Effect of water stress at different development stages on vegetative and reproductive growth of corn. **Field Crops Research**, Amsterdam, v. 89, n. 1, p. 1-6, 2004.

CANTARELLA, H. Calagem e adubação do milho. In: BÜLL, L.T.; CANTARELLA, H. (Ed.). **Cultura do milho: fatores que afetam a produtividade**. Piracicaba: POTAFOS, 1993. p. 148-196.

CARVALHO, M.A.C.; SORATTO, R.P.; ATHAYDE, M.L.F.; SÁ, M.E. Produtividade do milho em sucessão a adubos verdes no sistema de plantio direto e convencional. **Pesquisa Agropecuária Brasileira**, Brasília, v. 39, p. 47-53, 2004.

CASSMAN, K.G.; DOBERMANN, A.; WALTERS, D.T.; YANG, H. Meeting cereal demand while protecting natural resources and improving environmental quality. **Annual Review of Environment and Resources**, Palo Alto, v. 28, n. 1, p. 315-358, 2004.

CHAPUIS, R.; DELLUC, C.; DEBEUF, R.; TARDIEU, F.; WELCKER, C. 2012. Resiliencies to water deficit in a phenotyping platform and in the field: How related are they in maize? **European Journal of Agronomy**, Amsterdam, v. 42, p. 59–67, 2012.

CHENG, P.C.; PAREDY, D.R. Morphology and development of the tassel and ear. In: FREELING, M.; WALBOT, V. (Ed.). **The maize handbook**. New York: Springer-Verlag, 1994. chap. 3, p. 37-47.

CIAMPITTI, I.A.; VYN, T.J. A comprehensive study of plant density consequences on nitrogen uptake dynamics of maize plants from vegetative to reproductive stages. **Field Crops Research**, Amsterdam, v. 121, n. 1, p. 2-18, 2011.

_____. Physiological perspectives of changes over time in maize yield dependency on nitrogen uptake and associated nitrogen efficiencies: a review. **Crop Science**, Madison, v. 133, n. 11, p. 366-377, 2012.

_____. Grain nitrogen source changes over time in maize: a review. **Crop Science**, Madison, v. 53, p. 336-377, 2013.

CIAMPITTI, I.A., CAMBERATO, J.; MURRELL, S.T.; VYN, T.J. Maize nutrient accumulation and partitioning in response to plant density and nitrogen rate: I. Macronutrients. **Agronomy Journal**, Madison, v. 05, n. 3, p. 1-13, 2013.

CIAMPITTI, I.A.; ZHANG, H.; FRIEDEMANN, P.; VYN, T.J. Potential physiological frameworks for mid-season field phenotyping of final plant nitrogen uptake, nitrogen use efficiency, and grain yield in maize. **Crop Science**, Madison, v. 52, p. 2728-2742, 2012.

CIRILO, A.G.; ANDRADE, F.H. Sowing date and kernel weight in maize. **Crop Science**, Madison, v. 36, p. 325-331, 1996.

COELHO, A.M. **Balço de nitrogênio (15 N) na cultura do milho (Zea mays L.) em um Latossolo Vermelho Escuro fase cerrado**. 1987. 142 p. (Dissertação - Mestrado em Solos e Nutrição de Plantas) – Escola Superior de Agricultura, Lavras, 1987.

_____. **Nutrição e adubação do milho**. Sete Lagoas: Embrapa CNPMS, 2006. 10 p.

COELHO, A.M.; CRUZ, J.C.; PEREIRA FILHO, I.A. Rendimento do milho no Brasil: chegamos ao máximo? **Informações Agronômicas**, Piracicaba, n. 101, 2003.

COOPER, M.; GHO, C.; LEAFGREN, R.; TANG, T.; MESSINA, C. Breeding drought-tolerant maize hybrids for the US corn-belt: discovery to product. **Journal of Experimental Botany**, Oxford, 2014. Disponível em: <doi:10.1093/jxb/eru064. 2014>. Acesso em 22 jan. 2015.

DOURADO NETO, D.; FANCELLI, A.L. **Produção de milho**. Guaíba: Agropecuária, 2000. 360 p.

DOURADO NETO, D.D.; PALHARES, M.; VIEIRA, P.A.; MANFRON, P.A.; MEDEIROS, S.L.P.; ROMANO, M.R. Efeito da população de plantas e do espaçamento sobre a produtividade de milho. **Revista Brasileira de Milho e Sorgo**, Sete Lagoas, v. 2, n. 3, p. 63-77, 2003.

DWYER, L.M.; TOLLENAAR, M.; HOUWING, L. A nondestructive method to monitor leaf greenness in corn. **Canadian Journal of Plant Science**, Ottawa, v. 71, p. 505-509, 1991.

FAOSTAT. **Food and agriculture commodities production**. 2013. Disponível em: <<http://faostat.fao.org/>>. Acesso em: 10 mar. 2014.

FISCHER, K.S.; PALMER, A.F.E. Tropical maize. In: GOLDSWORTHYSY, P.R.; FISHER, N.M. (Ed.). **The physiology of tropical field crops**. Chichester: John Wiley, 1984. p. 213-248.

FRANCELLI, A.L.; DOURADO NETO, D. **Produção de milho**. 2. ed. Piracicaba: Livrocere, 2004. 360 p.

IGUE, K. Dinâmica da matéria orgânica e seus efeitos nas propriedades do solo. In: FUNDAÇÃO CARGILL. **Adubação verde no brasil**. Campinas, 1984. p. 232-267.

KINIRY, J.R.; RITCHIE, J.T. Shade-sensitive interval of kernel number of maize. **Agronomy Journal**, Madison, v. 77, n. 5, p. 711-715, 1985.

LANDRY, J.; DELHAYE, S. The Tryptophan contents of wheat, maize and barley grains as a function of nitrogen content. **Journal of Cereal Science**, London, v. 18, n. 3, p. 259-266, 1993.

LINDSEY, A.J.; THOMISON, P.R. Drought Tolerant Corn Hybrid Response to Nitrogen Application Rate. In: ASA, CSSA, & SSSA International Annual Meeting, 158-2, **Proceedings...** Long Beach: ASA, 2014.

LÓPEZ-BUCIO, J.; MARTÍNEZ, V. de la, O.; GUEVARA-GARCÍA, A.; HERRERA-ESTRELLA, L. Enhanced phosphorus uptake in transgenic tobacco plants that overproduce citrate. **Nat Biotechnol**, New York, v. 18, p. 450-453, 2000.

MADDONNI, G.A.; OTEGUI, M.E. Intra-specific competition in maize: early establishment of hierarchies among plants affects final kernel set. **Field Crops Research**, Amsterdam, v. 85, p. 1-13, 2004.

MONNEVEUX, P.; SANCHEZ, C.; BECK, D.; EDMEADES, G.O. Drought tolerance improvement in tropical maize source populations: evidence of progress. **Crop Science**, Madison, v. 46, n. 1, p.180-191, 2006.

MORADI, H.; AKBARI, G.A.; KHAVARI KHORASANI S.; RAMSHINI H.A. Evaluation of drought tolerance in corn (*Zea mays* L.) new hybrids with using stress tolerance indices. **European Journal of Sustainable Development**, Rome, v. 1, p. 543-560. 2012.

MUCHOW, R.C. Nitrogen utilization efficiency in corn and sorghum. **Field Crops Research**, Amsterdam, v. 56, p. 209-216, 1998.

NILSON, E.T.; ORCUTT, D.M. Water limitation. In: _____. **The physiology of plants under stress**. New York: Wiley, 1996.

OTEGUI, M.E.; ANDRADE, F.H.; SUERO, E.E. Growth, water use, and kernel abortion of maize subjected to drought at silking. **Field Crops Research**, Amsterdam, v. 40, n. 2, p. 87-94, 1995.

PANDA, R.K.; BEHERA, S.K.; KASHYAP, P.S. Effective management of irrigation water for maize under stressed conditions. **Agricultural Water Management**, Amsterdam, v. 66, n. 3, p. 181-203, 2004.

PANDEY, R.K.; MARANVILLE, J.W.; ADMOU, A. Deficit irrigation and nitrogen effects on maize in a Sahelian environment. I. Grain yield and yield components. **Agricultural Water Management**, Amsterdam, v. 46, n. 1, p. 1-13, 2000.

PEIXOTO, C.M. **Resposta de genótipos de milho à densidade de plantas, em dois níveis de manejo**. 1996. 118 p. Dissertação (Mestrado em Fitotecnia) - Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, 1996.

PEREIRA, R.G.; ALBUQUERQUE, A.W.; MADALENA, J.A.S. Influência dos sistemas de manejo do solo sobre os componentes de produção do milho e *Brachiaria decumbens*. **Revista Caatinga**, Mossoró, v. 22, n. 1, p. 64-71, 2009.

PEREIRA, R.S.B. Caracteres correlacionados com a produção e suas alterações no melhoramento genético do milho (*Zea mays* L.). **Pesquisa Agropecuária Brasileira**, Porto Alegre, v. 26, n. 5, p. 745-751, 1991.

PIEKIELEK, W.P.; FOX, R.H.; TOTH, J.D.; MACNEAL, K.E. Use of a chlorophyll meter at the early dent stage of corn to evaluate N sufficiency. **Agronomy Journal**, Madison, v. 87, n. 3, p. 403-408, 1995.

PIONNER. **Híbridos de milho: 30F35HR**. Santa Cruz do Sul, 2014. Disponível em: <<http://www.pioneersementes.com.br/DownloadCenter/Catalogo-De-Produtos-Milho-Safrinha-2014.pdf>>. Acesso em: 20 jan. 2015.

RIBEIRO, A.C.; GUIMARÃES, P.T.G.; ALVAREZ V., V.H. **Recomendação para o uso de corretivos e fertilizantes em Minas Gerais**. Viçosa: SBCS, 1999. 359 p.

RITCHIE, S.W.; HANWAY, J.J.; BENSON, G.O. Como a planta de milho se desenvolve. **Informações Agronômicas**, Piracicaba, n. 103, p. 1-11, 2003.

ROTH, J.A.; CIAMPITTI, I.A.; VYN, T.J. Physiological evaluations of recent drought tolerant maize hybrids at varying stress levels. **Agronomy Journal**, Madison, v. 5, n. 4, p. 1129-1141. 2013.

SABATA, R.J.; MASON, S.C. Corn hybrid interactions with soil nitrogen level and water regime. **Journal of Production Agriculture**, Madison, v.5, n. 1, p.137-142, 1992.

SANGOI, L. **An ideotype of maize for conditions of high temperature and low moisture.** 350 p. Dissertation (Ph.D. in Agronomy) - Iowa State University, Ames, 1996.

_____. Understanding plant density effects on maize growth and development: an important issue to maximize grain yield. **Ciência Rural**, Santa Maria, v.31, n.1, p.159-168, 2001.

SANGOI, L.; SALVADOR, R.J. Maize susceptibility to drought at flowering: a new approach to overcome the problem. **Ciência Rural**, Santa Maria, v. 28, n. 4, p. 699-706, 1998.

SANGOI, L., SILVA, P.R.F.; ARGENTA, G. Bases morfofisiológicas para maior tolerância dos híbridos modernos de milho a altas densidades de plantas. **Bragantia**, Campinas, v. 61, n. 2, p.101-110, 2002.

_____. **Estratégias de manejo do arranjo de plantas para aumentar o rendimento de grãos de milho.** Lages: Graphel, 2010. 64p.

SANGOI, L.; ERNANI, P.R.; LECH, V. A.; RAMPAZZO, C. Volatilização de N-NH₃ em decorrência da forma de aplicação de uréia, manejo de resíduos e tipo de solo, em laboratório. **Ciência Rural**, Santa Maria, v. 33, n. 4, p. 687-692, 2003.

SANGOI, L.; SILVA, P.R.F.; ARGENTA, G.; HORN, D. Bases morfo-fisiológicas para aumentar a tolerância de cultivares de milho a altas densidades de plantas. In: REUNIÃO TÉCNICA CATARINENSE DE MILHO E FEIJÃO, 4., 2003, Lages. **Resumos...** Lages: CAV-UDESC, 2003. p. 19-24.

SAS INSTITUTE. **SAS/STAT 9.1 User's Guide.** Cary: SAS Institute, 2004. 824 p.

SCHENK, M.K.; BARBER, S.A. Phosphate uptake by corn as affected by soil characteristics and root morphology. **Soil Science Society of America Journal**, Madison, v. 43, n. 4, p. 880-883, 1979.

SOUZA, C.M. **Efeito do uso contínuo de grade pesada sobre algumas características físicas e químicas de um Latossolo Vermelho-Amarelo Distrófico, fase cerrado, e sobre o desenvolvimento das plantas e absorção de nutrientes pela cultura de soja.** 1988. 105 p. Dissertação (Mestrado em Agronomia) - Universidade Federal de Viçosa, Viçosa, 1988.

STEWART, W.M.; DIBB, D.W.; JOHNSTON, A.E.; SMYTH, T.J. The contribution of commercial fertilizer nutrients to food production. **Agronomy Journal**, Madison, v. 97, n. 1, p. 1-6, 2005.

SWANK, J.C.; BELOW, F.E.; LAMBERT, R.J.; HAGEMAN, R.H. Interaction of carbon and nitrogen metabolism in the productivity of maize. **Plant Physiology**, Lancaster, v. 70, n. 4, p. 1185- 1190, 1982.

THOMAS, H.; HOWARTH, C.J. Five ways to stay green. **Journal of Experimental Botany**, Oxford, v. 51, p. 329-337, 2000. Disponível em : <doi:10.1093/jexbot/51.suppl_1.329>. Acesso em: 22 jan. 2015.

THOMAS, H.; SMART, C.M. Crops that stay green. **Annals of Applied Biology**, Chichester, v. 123, n. 1, p. 93-219, 1993.

TOKATLIDIS, I.S.; KOUTROUBAS, S.D. A review of maize hybrids' dependence on high plant populations and its implications for crop yield stability. **Field Crops Research**, Amsterdam, v. 88, n. 2/3, p. 103–114, 2004.

TOLLENAAR, M. Sink-source relationships during reproductive development in maize: a review. **Maydica**, Bergamo, v. 22, p. 49-75, 1977.

TOLLENAAR, M.; LEE, E.A. Strategies for enhancing grain yield in maize. In: JANICK, J. (Ed.). **Plant breeding reviews**. Hoboken: John Wiley, 2010. v. 4, p. 37-82.

TOLLENAAR, M.; WU, J. Yield improvement in temperate maize is attributable to greater stress tolerance. **Crop Science**, Madison, v. 39, n. 6, p. 1597–1604, 1999.

TRAORE, S.B., CARLSON, R.E., PILCHER, C.D., RICE, M.E. Bt and Non-Bt maize growth and development as affected by temperature and drought stress. **Agronomy Journal**, Madison, v. 92, n. 5, p. 1027–1035, 2000.

TROYER A.F.; ROSENBROOK, R.W. Utility of higher plant densities for corn performance testing. **Crop Science**, Madison, v. 23, n. 5, p. 863-867, 1983.

UHART, S.A.; ANDRADE, F.H. Nitrogen deficiency in maize: I. Effects on crop growth, development, dry matter partitioning, and kernel set. **Crop Science**, Madison, v. 35, n. 5, p. 1376-1383, 1995.

YAN W.; WALLACE D.H. Breeding for negatively associated traits. In: JANICK, J. (Ed.). **Plant breeding reviews**. Hoboken: John Wiley, 1995. v. 13, p. 141-177.

3 PHYSIOLOGICAL CHARACTERIZATION OF RECENT DROUGHT-TOLERANT MAIZE HYBRIDS AT VARYING STRESS LEVELS: PHOTOSYNTHESIS, GRAIN YIELD, AND GRAIN YIELD COMPONENTS

Abstract

Maize (*Zea mays* L.) breeding for drought tolerance have brought substantial progress, but little is known about the physiological mechanisms for commercial hybrids identified as possessing drought tolerance. The primary objective of this study was to investigate the physiological and yield responses of comparable-maturity drought and non-drought tolerant hybrids to varied plant density and N rates. The secondary objective was to specifically examine leaf photosynthesis and transpiration rates of hybrids across the growing season in response to varied PD and N rate treatments. The experiment was in northwest Indiana, USA. A five replicate split-split plot design was utilized with hybrid as main plot, plant density (PD) as the subplot, and N rate as the sub-subplots. Four hybrids were compared consisting of two pairings with different drought-tolerance: 111 (AQUAmax™ P1151 HR (Hybrid 1) versus P1162 HR (Hybrid 2), and 114 CRM hybrids (AQUAmax™ P1498 HR (Hybrid 3) versus 33D49 HR (Hybrid 4). The two PD levels were 78,000 (PD1) and 99,000 (PD2) plants ha⁻¹ final stand. All plots received 26 kg N ha⁻¹ in a 5-cm x 5cm starter band (19-17-0) at planting. Side-dress UAN (28-0-0) treatments of either 0 (Nr1), 134 (Nr2), 202 (Nr3), or 269 (Nr4) kg N ha⁻¹ were coulter-injected between corn rows. The following variables were analyzed: Photosynthesis (*A*), Transpiration (*E*) Leaf Area Index (LAI) and Grain Yield (GY) and its components. All hybrids responded similarly for GY to the treatment factors. Hybrid 3 (AQUAmax) seemed to have higher capacity of keeping stomata open, even in dry periods, that improved the hydraulic conductance in the plant, as evidenced by higher *E* (numeric number) than Hybrid 4 (non-drought tolerant, 33D49) at all sampling stages. Hybrid 3 resulted in lower LAI and higher *A* at R4 stage. Even with higher *E* than its comparison counterpart (Hybrid 4) the Hybrid 3 achieved a better water use efficiency than the non-drought tolerant, because of the lower LAI (lower area to loose water) and higher *A* per unit leaf area. The Hybrid 1 (AQUAmax P1151) demonstrated a similar *A* and *E* than its non-drought tolerant counterpart of similar maturity since Hybrid 2 (P1162) had a lower LAI (at the R2 and R3 stages) and a similar GY as Hybrid 1. The drought-tolerance mechanism that is most important with P1498 is less clear than for P1151, but P1498 seemed to be able to maintain higher leaf transpiration and photosynthesis rates than 33D49 during the grain-fill period (regardless of moisture stress variation during the grain fill period). So perhaps this hybrid simply has improved persistence in root water uptake late in the season. There was no single trait differentiation in photosynthesis or transpiration measurements. There was no evidence that AQUAmax hybrids were any different than non-AQUAmax hybrids in their response to N fertilizers, and there is no evidence that optimum N fertilizer rates would be any lower for these AQUAmax hybrids relative to the non-drought tolerant hybrids in this study.

Keywords: Mechanisms; Leaf area index; Water use efficiency

3.1 Introduction

Maize (*Zea mays* L.) is one of major cereals grown throughout the world, providing widely used products for food, feed and raw material for industry, mainly due to the amount and the nature of energy reserves accumulated in grains (WEILAND, 2006). This crop belongs to the group with C4 type photosynthesis metabolism type, which is characterized by high productive potential. Among the C4 plants, maize is in the group of species with greater use efficiency for solar radiation or quantum efficiency, mostly because of its leaf architecture (HATTERSLEY, 1984). However, despite the C4 advantage, maize is strongly negatively affected by the environmental stress such as those caused by drought and nutrient deficiencies.

The stress caused by low water availability (drought) is a major problem to agriculture, and the ability of plants to survive such stress is of paramount importance to the development of agribusiness in any country (SHAO et al., 2008). Drought stress is triggered by climatic, edaphic and agronomic factors. For example, drought stress can be exacerbated by high plant densities. The plant's susceptibility to drought stress varies in dependence on the degree of stress, different accompanying stress factors, plant species, and their developmental stages (DEMIREVSKA et al., 2009). Drought stress affects almost all aspects related to the crop development; it reduces the photosynthesis and chlorophyll per unit leaf area, but also reduces leaf area, wilting and curling of the leaves, and stomatal closure, and affects other factors such flowering, nutrient uptake and partitioning (HSIAO, 1973; SANCHEZ et al., 1983; BERGAMASCHI et al, 2006; JALEEL et al., 2009; XIA, 2012).

Even in climatically favorable years, if the water deficit occurs in critical period, which is about 1 week prior to and 2 weeks post silking, there may be reduction in the yield of maize (BÄNZIGER et al., 2000; BERGAMASCHI et al., 2004; TOLLENAAR; LEE, 2011). This critical period in maize unfortunately corresponds to the period between late June and late July in the Eastern Corn Belt when periodic drought is most likely to occur. Studies about drought tolerance in maize crop can bring improvements in growth and crop yield in regions with water limitations (LI; SPERRY; SHAO, 2009). The selection of cultivars with some tolerance or resistance to water stress is considered a cost-effective strategy of adaptation management in areas susceptible to drought (TURNER, 1991).

Substantial progresses in studies about maize breeding for drought tolerance have brought satisfactory results, generating several tolerant genotypes (CAMPOS et al., 2006; MONNEVEUX et al., 2006; HAMMER et al., 2009; COOPER et al., 2014). Gains in yield 8.9 and 1.9% under drought and non-drought conditions, respectively, for the non-transgenic

drought-tolerant vs. conventional hybrids were claimed by a privately owned seed company (PIONEER, 2013). However, little is known about the physiological mechanisms for drought tolerance (BANZIGER et al., 2002). Accordingly, there are very few public research publications focused in physiology that have investigated the recently released drought-tolerant hybrids (ROTH; CIAMPITTI; VYN, 2013). Plant tolerance to any individual stress also needs to be demonstrated in the context of associated stress factors. Thus, for example, tolerance to plant density stress in maize is very much related to the timing and intensity of water and nutrient stresses. High plant densities are much more likely to result in lower yields at below-optimum N rates (BOOMSMA et al., 2009; CIAMPITTI et al., 2013).

The primary objective of this study was to investigate the physiological and yield responses of comparable-maturity drought and non-drought tolerant hybrids (P1151 vs. P1162, and P1498 vs. 33D49) to varied plant density and N rates. The secondary objective was to specifically examine leaf photosynthesis (*A*) and transpiration (*E*) rates of hybrids across the growing season in response to varied PD and N rate treatments. The questions we want to answer with this study are: Are the drought-tolerant hybrids different from their comparable-maturity hybrid counterparts (without drought tolerance)? Which physiological mechanisms do the drought-tolerant hybrids have to be more efficient in water use?

3.2 Materials and Methods

3.2.1 Location and Experimental Design

The experiment was conducted over one growing season (2013) in northwest Indiana at the Pinney Purdue Agricultural Center (PPAC) (41° 26' 49" N, 86° 55' 42" W). The non-irrigated field experiment was established on the Tracy sandy loam (coarse-loamy, mixed mesic Ultic Hapludalfs). The preceding crop was soybean [*Glycine max* (L.) Merr.], and the tillage system employed was fall chisel plow and spring secondary tillage. The experiment was planted on May 1st.

A five replicate split-split plot design was utilized with hybrid as main plot, plant density (PD) as the subplot, and N rate as the sub-subplots. Four hybrids were compared consisting of two pairings with different drought-tolerance: 111 CRM hybrids (AQUAmax™ P1151 HR (Hybrid 1) versus P1162 HR (Hybrid 2), and 114 CRM hybrids (AQUAmax™ P1498 HR (Hybrid 3) versus 33D49 HR (Hybrid 4). Drought tolerance scores, as determined by DuPont Pioneer on a nine point scale (1 = low, 9 = high), for P1151 and P1498 were both 9, and for the hybrids less drought-tolerant, P1162 and 33D49, were 8 and 7, respectively.

Although the seeding rates were 79,000 and 104,000 plants ha⁻¹, the two actual PD levels were 78,000 (PD1) and 99,000 (PD2) plants ha⁻¹ final stand.

All plots received 26 kg N ha⁻¹ in a 5-cm x 5cm starter band (19-17-0) at planting. Side-dress urea ammonium nitrate, UAN, (28-0-0) treatments of either 0 (Nr1), 134 (Nr2), 202 (Nr3), or 269 (Nr4) kg N ha⁻¹ were coulter-injected between corn rows around the V5 growth stage (ABENDROTH et al., 2011). Intensive measurements were performed in three replicates whose individual plots measured 4.6 meters wide (six 76.2 cm rows) by 27 meters long (18 m long for the remaining two replicates). Soil was sampled from the 0 to 30cm layer, collecting 20 cores (2-cm diameter) from non-fertilized plots, before and after the side-dress N application. Soil samples for the standard soil-test fertility data were randomly collected by coring to the 20-cm depth in each replication; these samples were then analyzed by A&L Great Lakes Laboratories (Table 3.1). Soil cores for soil mineral N status were only taken from the 0N plots, again for each replication, close to the date of side-dress N application to a 30-cm depth. Data about weather conditions were recorded in both seasons (Figure 3.2).

Table 3.1 - Soil analysis for non-fertilized plots (inorganic nitrogen [NO₃⁻ - N / NH₄⁺ - N], organic matter content [OM], soil pH, potassium content [K], and phosphorus, Bray – P1 [P]) in 2013 season. At PPAC, Wanatah, IN, United States

Soil parameters	2013 season
NO ₃ ⁻ /NH ₄ ⁺ - N, mg kg ⁻¹	11.9 (3.10)/3.1 (0.54)
OM content, g kg ⁻¹	17.1 (0.33)
pH units	6.59
P, mg P kg ⁻¹	48.5 (11.13)
K, mg K kg ⁻¹	134.6 (25.19)

The value within parenthesis refers to standard deviation

3.2.2 Photosynthesis (A) and Transpiration (E) at the Leaf Level

A Li-Cor 6400XT – IRGA, Infra-Red Gas Analyzer (LI-COR, Lincoln, NE) was used to determine rates of photosynthesis (*A*, CO₂ exchange rate) and transpiration (*E*), leaf temperature, and vapor pressure deficit (VPD); all parameters determined at the leaf-scale. Measurements were taken at V10, V12, R2, R3, and R4 growth stages. Measurements were performed in the center two rows on all hybrids and PDs but only at two N rates (Nr1 vs. Nr3), in three replications for V10, V12, and R4 and only in two replications for R2 and R3. Two plants were measured per plot and two measurements were taken at the midpoint on each leaf (opposite the mid-rib).

During the vegetative phase the youngest collared leaf was selected, while the ear leaf was used during the reproductive period. An isolation technique was performed [XIA (2012), modified from Dwyer et al. (1995) and EARL and Tollenaar (1999)]. Representative plants were selected for measurements and all neighboring plants within 0.50-0.75 m were removed to eliminate shading. Plants were allowed to acclimate to the full sunlight for 1 hour prior to measurements with measurements occurring approximately between 11 a.m. and 3 p.m. Each replication required approximately 2.5 hours to complete all measurements.

In order to minimize environmental influence, measurements were taken on cloudless days and artificial light was used. Settings for the Li-Cor 6400XT were: i) photosynthetic photon flux density (PPFD) set at 2000 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$; ii) sample chamber CO_2 held constant by the CO_2 mixer at 400 $\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ air}$; and iii) flow set at 400 $\mu\text{mol air s}^{-1}$.

3.2.3 Leaf Area Index (LAI)

Leaf area index (LAI) was estimated by using the Li-Cor 2200 Plant Canopy Analyzer (LI-COR, Lincoln, NE). Three measurements were taken at ground level in the center rows, one at the $\frac{1}{4}$ row position, one at the $\frac{1}{2}$ row position, and one at the $\frac{3}{4}$ row position. Individual plot LAI values were means of these three measurements. The measurements were taken at V10, V12, R2, R3, and R4 development stages.

3.2.4 Flowering Development

The information gathered on 20 plants/plot at flowering included anther suspension and silk extrusion. Plots were monitored each day at the beginning of the tassel period (prior to silk extrusion for hybrids evaluated) and observations continued until all plants completed silking. Plants were counted as being at anthesis when at least 10 anthers were suspended from the tassel, and at silking when silks extruded at least 1 cm from the ear. A 10-90% anthesis (or silking) duration was calculated by subtracting the dates at which 2 plants (10%) had reached anthesis (or silking) from the date at which 18 plants (90%) had reached anthesis (or silking). The anthesis-silking interval (ASI) was calculated by subtracting the date at which 10 plants (50%) reached silking from the date at which 10 plants (50%) reached anthesis, since ASI is usually a positive number.

3.2.5 Grain Yield and Yield Components

A methodology identical to the one used by Roth et al. (2013) was followed. At maturity, GY and its components were determined in both seasons. Actual kernel number

(KN) per plant and kernel weight (KW) (adjusted to 155 g kg⁻¹) were determined from ears of the consecutive 20 plants ears from the center rows at R6 in three replications of each treatment. GY (also adjusted to 155 g kg⁻¹) were measured after harvesting the central two rows with a Kincaid XP plot combine.

3.2.6 Statistical Analyses

For statistical analyses, the hybrid treatment was treated as a fixed variable. The analysis of variance (ANOVA) was executed using SAS PROC MIXED (SAS INSTITUTE, 2004) and differences among treatment means were also analyzed. For photosynthetic parameters, the VPD factor was analyzed aiming to see if this is an influential and significant covariable.

3.3 Results and Discussion

3.3.1 Growing Season and Phenology

The weather conditions during the whole growth season can be seen in at Figure 3.2. The growing season experienced near normal temperatures and precipitation relative to historical trends at this location that can be reviewed at Ciampitti and Vyn (2011). Of most pertinence to this study was that there was little moisture stress during the critical period bracketing silking.

3.3.1.1 Anthesis-Silking Interval (ASI)

Because of generally favorable rain in early July (until July 11th), all four hybrids had good pollination conditions as well as a low anthesis to silking interval (Figure 3.1). The ASI (based on time from 50% pollen shed to 50% silk emergence) was as short as zero for Hybrid 2 (P1162), and was never more than 2 days for Hybrid 4 (P33D49). As anticipated from the comparison of 111 CRM versus 114 CRM hybrid pairs, hybrids P1151 and P1162 had early flowering in relation to P1498 and 33D49. Is important to comment that the Hybrid P1498 achieved a shorter ASI than Hybrid 33D49. The ASI trait is widely recognized as an important indicator of the sensitivity tolerance of maize to water deficits that coincide with the flowering period. Under water deficit at flowering, a shorter ASI indicates better potential to avoid or tolerate the effects of water deficits and a higher ASI values indicates greater sensitivity and intolerance to the effects of water deficit (COOPER et al., 2014).

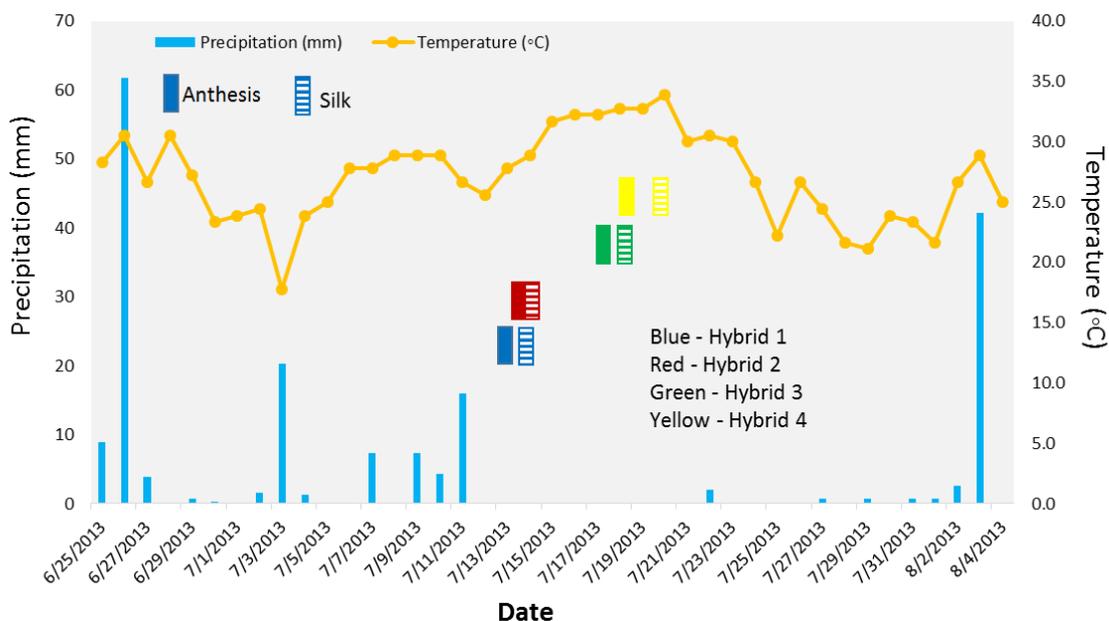


Figure 3.1 - Timing of anthesis and silk emergence (both shown the day of 50%), for four hybrids (Hybrid 1 = AQUAmax™ P1151, Hybrid 2 = P1162, Hybrid 3 = AQUAmax™ P1498, Hybrid 4 = 33D49) averaged across each plant density level (PD1 = 78,000, and PD2 = 99,000 pl ha⁻¹), and averaged across all four N rate levels (Nr1 = 0, Nr2 = 134, Nr3 = 202, and Nr4 = 269 kg N sidedress ha⁻¹) as related to maximum air temperature and precipitation before and after the flowering period, 2013 season

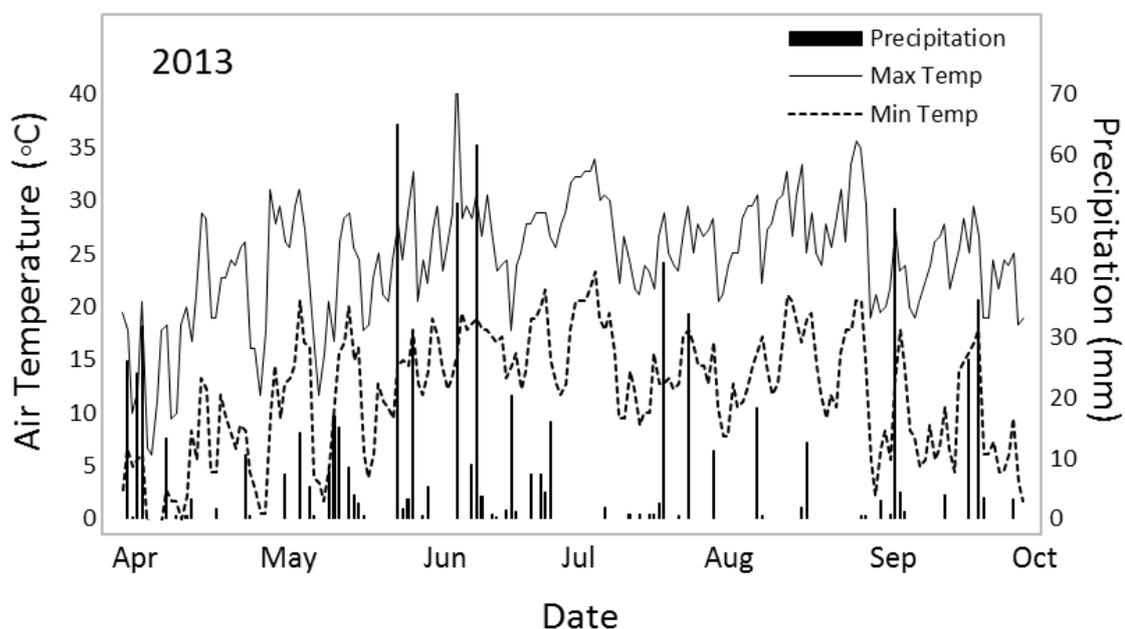


Figure 3.2 - Weather conditions (maximum and minimum air temperature and mean precipitation) for 2013 maize growing seasons at Pinney-Purdue Agricultural Center (PPAC) in northwestern Indiana, USA

3.3.2 Leaf area index

The effects of hybrid, plant density, nitrogen rates, stage of development, and the Hybrid x Stage interaction all resulted in significant differences for LAI (Table 3.2; Figure 3.3). The Hybrid 2 (P1162) had higher mean LAI than all other hybrids when averaged over the 5 measurement dates as well as for the R2 growth stage itself. Hybrid 1 (P1151) had lower LAI than its similar-maturity counterpart (P1162) all season. Hybrid 3 (1498) had fairly similar LAI to its similar-maturity counterpart Hybrid 4 (33D49) except at the R3 stage, especially at the higher plant density, when P1498 had higher LAI than 33D49. This short-term advantage for P1498 did not persist through the end of grain filling since P1498 had the lowest LAI value of all hybrids at the R4 stage. Also a significant factorial between PD x Nr at V12 stage was observed (Figure 3.8); the treatments with sidedress N (at all rates of 134 kg N ha⁻¹ or above) caused higher LAI in PD2 than in PD1, while in the zero N treatment there was no difference for LAI between the plant densities. After analyzing the N rates in the plant densities, there was only difference between N fertilized versus the non-fertilized treatments (Nr1 < Nr2=3=4) in both PD treatments. Hybrids achieved higher LAI values for PD2 at R2 and R3 stages with a decrease of LAI at the R4. These interaction relationships are presented for Hybrid x PD (Figure 3.6) and for Hybrid x Nr (Figure 3.7), the latter without a mean separation test.

In general, earlier maturing (lower CRM) hybrids, with lower demand in thermal time for flowering, had less leaf area per plant and less crop canopy shading, and therefore require higher plant density compared to normal cycle hybrids to achieve their yield potential (SANGOI, 2000). Abortion of ovules, kernels, and ears can occur from one week before to two weeks after silking. Drought, shade, high density, and/or N deficiency stress during this period accentuate these processes (UHART; ANDRADE, 1995).

3.3.3 Overall Photosynthesis (*A*) and Transpiration (*E*) Patterns

Photosynthetic (*A* - CO₂ exchange rate) and transpiration (*E*) rates are presented in Tables 1.3 and 1.4, respectively. The *A* at leaf and canopy scales declined progressively during the growing season following the leaf senescence process (Figures 3.3 and 3.4). Several studies have reported the same situation for the *A* declination during the growing season in field-grown maize (KIM et al., 2006; LEAKEY et al., 2006; ECHARTE et al, 2008; MARKELZ; STRELLNER; LEAKEY, 2011; XIA, 2012; ROTH et al., 2013). Significant decreases in *A* (after V10) and in *E* (after V12) for all hybrids were observed. Roth et al (2013), in a similar study, observed that both heat and drought stresses substantially reduced

A and *E* during the late vegetative-phase (V12, V15 and R1) that resulted in lower total plant biomass (BM) at the same location in 2012.

In this study, the green LAI declined by 50% or more from R2 to R4 growth stages (Table 3.2). The decrease of *A* and *E* per unit leaf area were a result more related to progressively later development stages (leaf senescence) than to the temperature responses. Newer hybrids maintain higher plant and grain biomass under N deficiency because their photosynthetic capacity decreases more slowly after anthesis than in older hybrids (DING et al., 2005).

Extending the duration of active photosynthesis can elevate the yield of crops and delaying leaf senescence is one of the ways to accomplish this (SPANNO et al., 2003). Ma and Dwyer (1998) concluded that a maize hybrid with a long duration of active photosynthesis produced 24% more dry matter and assimilated 20% more N than a quick-leaf-senescence hybrid during grain filling stage. However, the photosynthesis in leaves, especially in leaves of quick-leaf-senescence genotypes, begins to decrease during the later developmental stages, which severely limits grain yield (TOLLENAAR; DAYNARD, 1978).

3.3.4 Growth Stage Evaluation: Photosynthesis (A) and Transpiration (E) per unit Leaf Area

In analyzing the Figures 3.4, 3.5, 3.9 and 3.10 it is clear that Hybrid 1 (AQUAmax P1151) has consistently lower Photosynthesis (*A*) and Transpiration (*E*) than its counterpart (Hybrid 2, P1162); this trend was more evident from V12 to R3 stages. However at R4 most *A* values had a tendency to be equalized. Nevertheless the Hybrid 3 (AQUAmax P1498) showed an overall higher *A* and *E* than the Hybrid 4 (33D49). There was a stronger difference for both *A* and *E* values in the PD1 than in the PD2 at the R2 stage (this stage happened after July 12th). In July a deficiency of precipitation after the 11th day was observed; consequently, there was less water availability in the soil from this stage until the beginning of August. Even with this water deficiency, from mid-July to early August, the Hybrid 3 exhibited a higher *A* and numerically *E* than the Hybrid 4. Thus, Hybrid 3 could have a mechanism of keeping open stomata even in the situation with lower water availability in the soil that could improve hydraulic conductance in the plant. Stomatal response is considered to be related to the hydraulic dynamics in the plant. High water vapor loss from guard cells and/or a shortage of water supply to the epidermal cells, including the guard cells in particular, can result in decreased turgor pressure and cause partial stomata closure (EAMUS et al., 2008). Transpiration restriction by stomata at high vapor pressure deficit (VPD) appears to result

from limiting hydraulic conductance in the plant, which constrains the flow of water from roots to transpiration sites in the leaf surface (SINCLAIR; ZWIENIECKI; HOLBROOK, 2008; SADOK; SINCLAIR, 2010).

Similar air temperatures during the photosynthesis measurement periods from V10 to R2 were observed with a value close to 33 °C; however at the R3 sampling date a decrease of air temperature was observed (mean of 29.3°C) and at R4 there was once again a higher mean air temperature averaging 34.2°C (Table 3.3). These changes in the air temperature, plus the resumption of precipitation just prior to R4, could explain the increase from R3 to R4 of the *E* after subsequent decreases from V12 to R3. However, numerically, the *A* of AQUAmax hybrids did not strongly decrease as much as the non-AQUAmax hybrids did. The values for *A* (micromol CO₂ m⁻²s⁻¹) in R3 and R4 were, respectively: Hybrid 1 (22.9 and 23.3) - Hybrid 2 (27.2 and 23.2) and Hybrid 3 (28.3 and 26.8) – Hybrid 4 (23.1 – 22.2), the *A* of Hybrid 2 was the most negatively affected by the combination of the later development stage with higher air temperature in the first pair of similar-maturity hybrids (P1151 and P1162) when there was a decrease of almost 4 (micromol CO₂ m⁻²s⁻¹) in the *A* value. However, even though Hybrid 3 (P1498) had a small decrease of *A*, the *A* value of this hybrid still was very much higher than its pair of comparison with the non-AQUAmax Hybrid 4 (33D49) that also showed a decline in the *A* value from R3 to R4. Photosynthesis is usually inhibited when leaf temperature exceeds about 38°C (BERRY; BJÖRKMAN, 1980; EDWARDS; WALKER, 1983). Decreased leaf photosynthesis at high temperatures is principally due to reduced efficiency in photosystem II, rather than an increase in maintenance dark respiration or a decrease in leaf area (PRANGE et al., 1990).

Therefore, the higher *E* during the dry period could be a water deficiency tolerance mechanism caused by a more continuous open stomata pattern. As a result of this, high photosynthesis rates were maintained longer for Hybrid 3. Nonetheless, statistically there was only a difference for *E* in the V12 stage (Table 3.4), but the pairs of hybrids did not differ between each other (Hybrid 1 = 2; Hybrid 3 = 4). Similar results for *A* in V10 was observed (Hybrid 1 = 2; Hybrid 3 = 4), Table 1.3. Nevertheless in R4 stage the Hybrid 3 exhibited a higher *A* value than Hybrid 4 (with a difference of the 5 micromol Co₂ m⁻²s⁻¹). The PD treatment factor was not significant for *A* nor for *E*. While the N rate caused a significant difference for both, the *A* and *E* were greater in the plots with N treatment (Nr3) in comparison to without N application in sidedressing. There were no significant interactions between Hybrid and PD nor for Hybrid x Nr in all sampling growth stages. Similar results were observed by Roth et al. (2013) in a similar research across two seasons (2011 and 2012).

They observed minimal hybrid differences in *A* and *E*, and also for GY. In this study the N rate also was the primary factor governing *A* and *E* responses during late drought periods. The higher N rate had a positive impact on *A* and *E* under adequate moisture, but a minor negative effect during drought conditions.

The lower LAI with Hybrid 3 at R4 stage, in comparison with Hybrid 4, in addition with the maintenance of higher photosynthesis and transpiration rates could be traits that confer drought stress tolerance. Because even with higher transpiration this hybrid had lower leaf area to experience water loss. Additionally, the transpiration is a factor totally related to the maintaining photosynthesis capacity. Both long- and short-term water deficit resources lead to many physiological alterations. Long-term drought response includes altered root to shoot ratio (BLUM; ARKIN, 1984), reduced leaf area; and short-term drought responses include altered osmotic adjustment (TURNER et al., 1986).

3.3.5 Total Plant Biomass, and Grain Yield and Its Components

There were significant responses for both hybrid and N rate treatment factors for total plant biomass (BM), but not for the PD factor. The BM responded progressively higher as N rates increased. Hybrid 2 achieved the highest total plant biomass; there were no statistical total biomass difference among other hybrids, but only between them and Hybrid 2 (Table 3.5).

Significant differences ($P < 0.05$) in KN and KW were evident in all three treatment factors (hybrid, plant density and N rate), Table 3.5. Hybrids P1151 and P1162 had the highest KW values, but the lowest KN values, while hybrid 33D49 had just the opposite grain component responses. As expected, both mean KN and mean KW across the 4 maize hybrids were higher at the low density and as N rates increased, although the N rate of 269 Kg per hectare of N did not significantly increase either KN or KW relative to the 202 kg N rate.

The drought-tolerant hybrids (AQUAmax) revealed higher grain harvest index (GHI) in comparison to non-drought tolerant hybrids (Hybrid 1 > Hybrid 2; Hybrid 3 > Hybrid 4) (Table 3.5). The higher PD negatively affected the GHI (PD1 > PD2). The higher N rates increased GHI; the actual GHI gain between N rate 4 (269 kg N ha⁻¹) and N rate 1 (zero N) averaged 0.10 (or a full 10% of the total BM).

Grain yields are first presented as mean yields averaged over plant densities and N rates (Table 3.5). Overall grain yields were very high in this experiment. Plot-combine harvested grain yields were statistically different ($P < 0.05$) for hybrid and N rate treatment factors (Table 3.5; Figure 3.11); the same treatment factors were also significantly different for other variables. We observed that hybrid P1151 did not differ from P1162, and only

differed from Hybrid 33D49. Hybrid 33D49 had the lowest grain yield, and this hybrid was also significantly lower in yield than its comparable-maturity counterpart with the drought-tolerance trait (P1498). There was a huge response to N rates. Each increment of N fertilizer increased yields significantly, and the N rate 4 (269 kg N per hectare) resulted in an average yield increase of 7.7 Mg per hectare compared to the control.

Of greatest interest in this study is the relative comparison of the AQUAmax hybrids versus comparable-maturity conventional hybrids. All hybrids, whether labeled as more drought tolerant or less so, responded fairly similarly in grain yield to plant density and N rate treatments. Specific AQUAmax hybrids vary considerably in their physiology differences with comparable-maturity hybrids, but did not demonstrate improved grain yields or more yield stability than non-AQUAmax hybrids when grown at high plant density (99,000 plants per hectare) than at the standard plant density (78,000 plants per hectare), or when grown with a N deficiency stress. The AQUAmax hybrid P1151 generally displayed lower LAI during the reproductive period, a longer anthesis to silking interval, as well as lower leaf photosynthesis and leaf transpiration rates during the entire growing season, than comparable-maturity hybrid P1162. These results confirm that P1151 seems to have a root-zone water conservation strategy relative to other commercial hybrids that would benefit corn plants in certain persistent drought environments. The benefits of a water-conserving strategy would be most likely if drought symptoms were severe during the reproductive period. AQUAmax hybrid P1498 generally showed approximately equal LAI, a slightly earlier flowering, a lower anthesis-to-silking interval, and similar to higher leaf photosynthesis and leaf transpiration rates than comparable-maturity hybrid 33D49. Hybrid 1498 also had consistently lower kernel numbers but higher final kernel weights than 33D49. Grain yields were higher with P1498, perhaps because 33D49 was the last hybrid to flower in the middle of a 3-week period with no rain (July 12 to August 1, 2013).

3.3.6 Discussion

Roth, Ciampitti and Vyn (2013), in a similar study, in two grown seasons (2011 and 2012) with same hybrids (Hybrid 1 - P1151 and Hybrid 2 - P1162, in 2011) and (Hybrid 1 - P1151, Hybrid 2 - P1162, Hybrid 3 - P1498, and Hybrid 4 - 33D49, in 2012), at the same all 4 N rates, and similar two PD, evaluated *A* and *E* at R3, R4, and R5 (in 2011) and at V10, V12, V15, R1, R3, R4, and R5 (in 2012). They reported above-normal temperatures and record of drought conditions with only 61 mm of rain from 1st June to mid-July in 2012 season, and near normal weather conditions in 2011. The weather conditions in 2012 resulted in severe

plant stress most evident from V12 to R1. In this study, N rate significantly affected *A* only at R4 and R5 stages. As the same way in our study, that presented more normal weather conditions in comparison with 2012, related to historical climatological trend by made CIAMPITTI and VYN (2011), the N rate was significant at R4, however was also significant at earlier stages V10 and V12. The lack of N rate effect at earlier stages in 2012 season might be associated with the interaction between N and low water supply. Additionally in this study, N rates rather than the hybrid or PD, was the mainly factor governing *A* and *E* in both seasons (ROTH, CIAMPITTI; VYN, 2013). Therefore, in our study there was a statistical significant difference for the hybrid factor, Hybrid 3 (AQUAmax P1498) showed an overall higher *A* and *E* than the Hybrid 4 (33D49), thus the first one presented to be more efficient in water use than the Hybrid 4. Bunce (2010, 2011) observed significant differences in *E* for maize inbreds and hybrids. Genetic variation at the inbred level was recently documented by Benešova et al. (2012) with major improvements in *A* for some inbreds under drought conditions but with lesser variation in *E*.

3.4 Conclusions

The N rate, rather than hybrid or PD, was the primary factor governing leaf *A* and *E* responses during the different development stages. In summary, all hybrids whether labeled as more drought-tolerant or less so, responded similarly (for GY and some parameters measured) to PD and N treatment factors. Hybrid differences in *A* and *E* per unit leaf were not found across a range of simultaneous stress conditions arising from low N or high PD. However the Hybrid 2 achieved higher BM than Hybrid 1 and also than all hybrids in this study. Nevertheless, the actual LAI attained and retained was the most important factor in biomass and yield formation.

The Hybrid 1 (AQUAmax P1151) never achieved a higher *A* and *E* than its non-drought tolerant counterpart of equal maturity Hybrid 2 (P1162). Hybrid 1 had a lower LAI (at the R2 and R3 stages) than the second hybrid and a similar GY. Hybrid 3 (AQUAmax P1498) had a capacity of keeping stomata open, even in the dry stage, that improved the hydraulic conductance in the plant, as evidenced by the higher *E* (numeric number) than Hybrid 2 (non-drought tolerant) in all sampling periods. Additionally, Hybrid 3 presented lower LAI and higher *A* at R4 stage. Even with higher *E* than its pair of comparison, Hybrid 4, the Hybrid 3 presented a better water use efficiency than the non-drought tolerant hybrid 4, perhaps because of a lower LAI (lower area to loose water) and higher *A*.

The drought-tolerance mechanism that is most important with P1498 is less clear than for P1151, but P1498 seemed to be able to maintain higher leaf transpiration and photosynthesis rates than 33D49 in the grain-fill period (regardless of moisture stress variation during the grain fill period). So perhaps this hybrid simply has improved persistence in root water uptake late in the season. There was no single trait differentiation in photosynthesis or transpiration measurements among these hybrids during the 2013 growing season.

There was no evidence that AQUAmax hybrids were any different than non-AQUAmax hybrids in their response to N fertilizers or to PD. Thus it is unlikely that N fertilizer management should change when AQUAmax hybrids are grown or that the AQUAmax hybrids could perform better at the higher plant density. Certainly there is no evidence that optimum N fertilizer rates would be any lower for these AQUAmax hybrids.

3.5 Future Research

The search for maize genotypes more tolerant to abiotic stresses is and will be a constant task in order to get higher grain yields in maize. As the same, periodic or persistent drought stress during growing season is and will continue be a frequent negative factor in maize crop production. Thus, companies will be working diligently towards the goal of having tolerant genotypes to diverse kinds of abiotic stresses, but with a priority on achieving better yields when to water deficiency occurs in the soil.

Accordingly, new studies with an even wider range of hybrids should continue to evaluate traits or mechanisms that makes maize crop tolerant to varying drought stress at different growth stages. The further continued research should have more seasons of evaluation that can be compared to each other to investigate crop responsiveness in different environments. Also is important that the evaluations be made during all the growing season, including the early and late (senescence) stages, preferably by aiming to study maize crop response related to the environment from germination until the physiological maturity. Another important factor is to conduct more plant evaluations below ground, these studies are very important to see how root patterns and root activity in different genotypes affect water use efficiency. Also is important to conduct evaluation of photosynthesis and transpiration during the different times of the day aiming to see the maize behavior with more variation of light and temperature resources during the diurnal cycles as the season progresses.

Table 3.2 - Leaf area index (LAI) ($\text{m}^2 \text{m}^{-2}$) obtained using Licor 2200 (LI-COR, Lincoln, NE) for all hybrids (Hybrid 1 = AQUAmaxTM P1151, Hybrid 2 = P1162, Hybrid 3 = AQUAmaxTM P1498, Hybrid 4 = 33D49) at both densities (PD1 = 78,000 pl ha⁻¹, PD2 = 99,000 pl ha⁻¹), and at four N rates (Nr1 = 0 kg N ha⁻¹, Nr2 = 134 kg N ha⁻¹, Nr3 = 202 kg N ha⁻¹, Nr4 = 269 kg N ha⁻¹) at various growth stages throughout the 2013 season. Mean values represent the average of 3 replications of 3 measurements per plot

2013	LAI ($\text{m}^2 \text{m}^{-2}$)					Mean
	V10	V12	R2	R3	R4	
Hybrid						
Hyb 1	2.2 ab	3.0	3.8 c	3.2 d	1.9 a	2.81 b
Hyb 2	2.3 a	3.2	4.9 a	4.6 a	2.0 a	3.42 a
Hyb 3	2.1 b	2.9	4.2 b	4.1 b	1.2 b	2.89 b
Hyb 4	1.8 c	2.9	4.1 bc	3.4 cd	2.0 a	2.83 b
PD						
PD 1	2.0 b	2.8 b	3.9 b	3.6 b	1.7	2.79 b
PD 2	2.2 a	3.3 a	4.5 a	4.0 a	1.8	3.18 a
Nr						
Nr 1	1.9 b	2.5 b	3.3 c	2.9 c	1.5 b	2.39 c
Nr 2	2.2 ab	3.2 a	4.3 b	2.7 b	1.6 b	3.02 b
Nr 3	2.1 a	3.2 a	4.8 a	4.2 a	2.1 a	3.28 a
Nr 4	2.1 a	3.2 a	4.5 ab	4.3 a	1.9 ab	3.22 ab
Anova						
Hyb	**	ns	**	**	**	*
PD	**	**	**	ns	ns	**
Nr	**	**	**	*	*	**
Hyb x PD	ns	ns	ns	ns	ns	ns
Hyb x Nr	ns	ns	ns	ns	ns	ns
PD x Nr	ns	*	ns	ns	ns	ns
Hyb x PD x Nr	ns	ns	ns	ns	ns	ns

ns = not significant; *=P<0.05; **=P<0.01; ***=P<0.0001

Table 3.3 - Leaf photosynthesis (A) (micromol CO₂ m⁻²s⁻¹) using Licor 6400XT (LI-COR, Lincoln, NE) for all hybrids (Hybrid 1 = AQUAmaxTM P1151, Hybrid 2 = P1162, Hybrid 3 = AQUAmaxTM P1498, Hybrid 4 = 33D49) at both densities (PD1 = 78,000 pl ha⁻¹, PD2 = 99,000 pl ha⁻¹) and at two N rates (Nr1 = 0 kg N ha⁻¹, Nr3 = 202 kg N ha⁻¹) at various growth stages throughout the 2013 season. Each value represents three replications (total 4 points per plot) from field isolated, light acclimated plants on the youngest fully expanded leaf at vegetative stages and the ear leaf at reproductive stages

AT(°C)#	Photosynthesis (A)				
	V10 (33.1)	V12 (33.9)	R2 (33.8)	R3 (29.3)	R4 (34.2)
Hybrid					
Hybrid 1	48.5 a	41.3	21.4	22.9	23.3 ab
Hybrid 2	49.1 a	41.7	27.9	27.2	23.2 ab
Hybrid 3	48.1 ab	39.8	30.7	28.3	26.8 a
Hybrid 4	44.2 b	40.1	24.9	23.1	22.2 b
PD					
PD1	48.0	41.3	26.8	26.2	25.8
PD2	46.8	40.2	25.6	24.6	21.8
Nr					
Nr1	44.5 b	36.2 b	25.2	21.5	21.6 b
Nr3	50.4 a	44.9 a	27.2	29.3	26.0 a
ANOVA					
Hyb	**	ns	NA	NA	**
PD	ns	ns	NA	NA	ns
Nr	**	**	NA	NA	**
Hyb x PD	ns	ns	NA	NA	ns
Hyb x Nr	ns	ns	NA	NA	ns
PD x Nr	ns	ns	NA	NA	ns
Hyb x PD x Nr					
Nr	ns	ns	NA	NA	ns

#AT = Mean air temperature in each sample stage in °C.

NA = not applicable; ns = not significant; * = P < 0.05; ** = P < 0.01; *** = P < 0.0001.

Only 2 reps were completed for R2 and R3 stage measurements and treatments could not be evaluated statistically

Table 3.4 - Leaf transpiration (E) ($\mu\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$) using Licor 6400XT (LI-cor, Lincoln, NE) for all hybrids (Hybrid 1 = AQUAmax™ P1151, Hybrid 2 = P1162, Hybrid 3 = AQUAmax™ P1498, Hybrid 4 = 33D49) at both densities (PD1 = 78,000 pl ha⁻¹, PD2 = 99,000 pl ha⁻¹) and at two N rates (Nr1 = 0 kg N ha⁻¹, Nr3 = 202 kg N ha⁻¹) at various growth stages throughout the 2013 season. Each value represents three replications (total 4 points per plot) from field isolated, light acclimated plants on the youngest fully expanded leaf at vegetative stages and the ear leaf at reproductive stages

	Transpiration (E)				
	V10	V12	R2	R3	R4
Hybrid					
Hybrid 1	8.04	8.18 ab	3.81	3.79	4.72
Hybrid 2	8.53	8.85 a	5.65	5.09	5.39
Hybrid 3	8.07	7.31 ab	5.52	4.46	4.92
Hybrid 4	7.61	7.00 b	4.32	3.44	4.17
PD					
PD1	8.12	7.97	4.91	4.37	5.07
PD2	8.00	7.77	4.74	3.98	4.49
Nr					
Nr1	7.47 b	6.86 b	4.81	3.68	4.64
Nr3	8.63 a	8.82 a	4.85	4.66	4.93
ANOVA					
Hyb	ns	**	NA	NA	ns
PD	ns	ns	NA	NA	ns
Nr	**	**	NA	NA	ns
Hyb x PD	ns	ns	NA	NA	ns
Hyb x Nr	ns	ns	NA	NA	ns
PD x Nr	ns	ns	NA	NA	ns
Hyb x PD x Nr	ns	ns	NA	NA	ns

ns = not significant; *=P<0.05; **=P<0.01; ***=P<0.0001.

NA = not applicable; ns = not significant; *=P<0.05; **=P<0.01; ***=P<0.0001.

Only 2 reps were completed for R2 and R3 stage measurements and treatments could not be evaluated statistically

Table 3.5 - Grain yield (155 g kg⁻¹ moisture) from combine harvest (GY), grain harvest index (GHI), kernel number (KN), kernel weight (KW), and total plant biomass, BM (Mg ha⁻¹) at physiological maturity for all maize hybrids (Hybrid 1 = AQUAmaxTM P1151, Hybrid 2 = P1162, Hybrid 3 = AQUAmaxTM P1498, Hybrid 4 = 33D49, and Hybrid 5 = P1184) grown at two plant densities (PD1 = 78,000, PD2 = 99,000 pl ha⁻¹) and four N rates (Nr1 = 0, Nr2 = 134, Nr3 = 202, and Nr4 = 269 kg ha⁻¹) in 2013

	GY (Mg ha ⁻¹)	GHI	KN (kernel m ⁻²)	KW (mg kernel ⁻¹)	Total BM (Mg ha ⁻¹)
Hybrid					
Hyb 1	13.24 a	0.55 a	4074 c	295 a	20.7 b
Hyb 2	12.96 a	0.51 bc	3827 d	304 a	22.0 a
Hyb 3	12.68 a	0.52 b	4522 b	249 b	20.0 b
Hyb 4	11.56 b	0.50 c	4839 a	227 c	19.6 b
PD					
PD 1	12.61	0.52 a	4196 b	274 a	20.4
PD 2	12.71	0.51 b	4435 a	263 b	20.8
Nr					
Nr 1	7.44 d	0.45 c	2773 c	247 c	13.8 c
Nr 2	13.38 c	0.52 b	4672 b	265 b	21.8 b
Nr 3	14.46 b	0.54 ab	4898 ab	278 a	23.1 ab
Nr 4	15.14 a	0.55 a	4919 a	286 a	23.6 a
Anova					
Hyb	**	**	**	**	**
PD	ns	**	**	**	ns
Nr	**	**	**	**	**
Hyb x PD	ns	ns	ns	ns	ns
Hyb x Nr	ns	ns	ns	ns	ns
PD x Nr	ns	ns	ns	ns	ns
Hyb x PD x Nr	ns	ns	ns	ns	ns

ns = not significant; *=P<0.05; **=P<0.01; ***=P<0.001

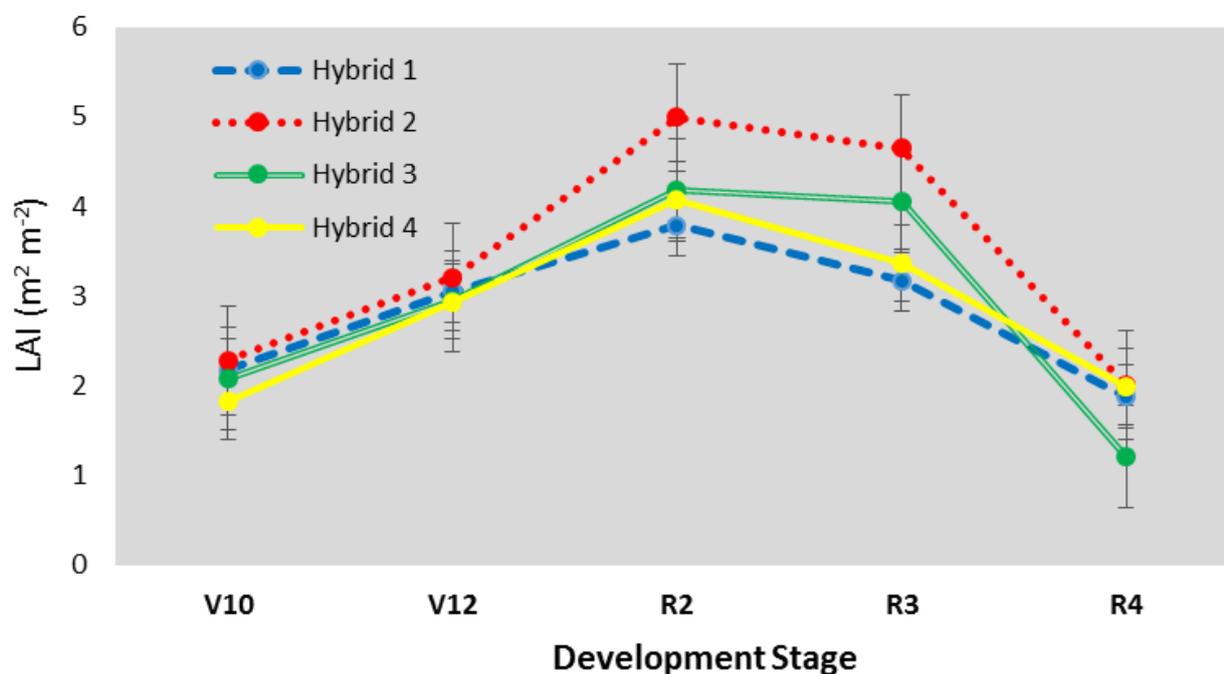


Figure 3.3 - LAI – Leaf Area Index ($\text{m}^2 \text{m}^{-2}$) for all hybrids (Hybrid 1 = AQUAmax™ P1151, Hybrid 2 = P1162, Hybrid 3 = AQUAmax™ P1498, Hybrid 4 = 33D49) at both densities ($\text{PD1} = 78,000 \text{ pl ha}^{-1}$, $\text{PD2} = 99,000 \text{ pl ha}^{-1}$) and at four N rates ($\text{Nr1} = 0 \text{ kg N ha}^{-1}$, $\text{Nr2} = 134 \text{ kg N ha}^{-1}$, $\text{Nr3} = 202 \text{ kg N ha}^{-1}$, $\text{Nr4} = 269 \text{ kg N ha}^{-1}$) at various growth stages throughout the 2013 season. Mean values represent the average of 3 replications of 3 measurements per plot

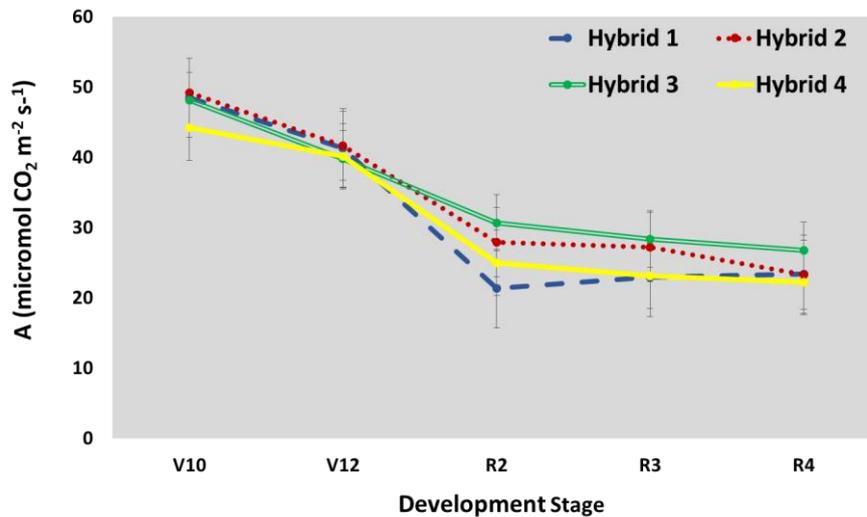


Figure 3.4 - A – Photosynthesis rates ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) using Licor 6400XT (LI-COR, Lincoln, NE) for all hybrids (Hybrid 1 = AQUAmax™ P1151, Hybrid 2 = P1162, Hybrid 3 = AQUAmax™ P1498, Hybrid 4 = 33D49) at both densities (PD1 = 78,000 pl ha⁻¹, PD2 = 99,000 pl ha⁻¹) and at two N rates (Nr1 = 0 kg N ha⁻¹, Nr3 = 202 kg N ha⁻¹) at various growth stages throughout the 2013 season. Each value represents three replications (total 4 points per plot) from field isolated, light acclimated plants on the youngest fully expanded leaf at vegetative stages and the ear leaf at reproductive stages. Note: Only 2 reps were completed for R2 and R3 stage measurements

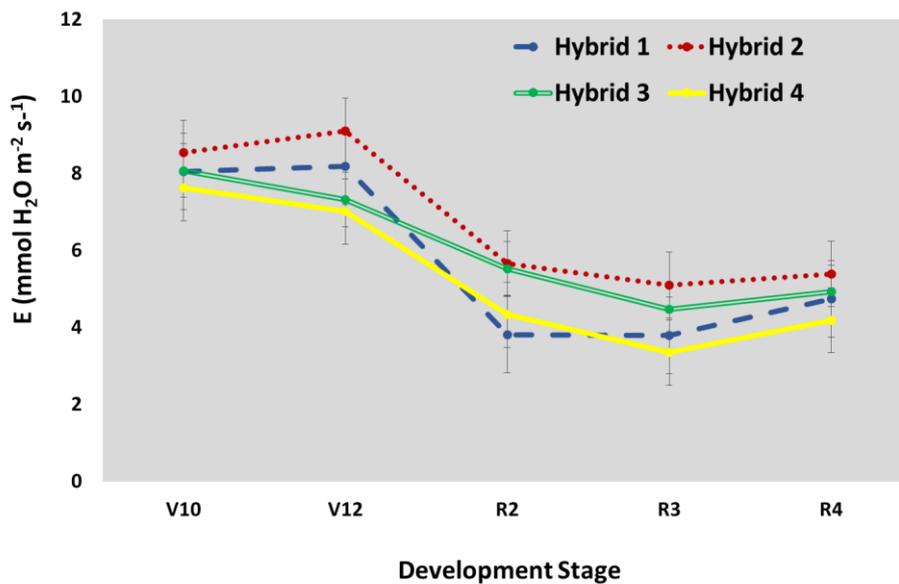


Figure 3.5 - E – Leaf transpiration rates ($\mu\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$) using Licor 6400XT (LI-COR, Lincoln, NE) for all hybrids (Hybrid 1 = AQUAmax™ P1151, Hybrid 2 = P1162, Hybrid 3 = AQUAmax™ P1498, Hybrid 4 = 33D49) at both densities (PD1 = 78,000 pl ha⁻¹, PD2 = 99,000 pl ha⁻¹) and at two N rates (Nr1 = 0 kg N ha⁻¹, Nr3 = 202 kg N ha⁻¹) at various growth stages throughout the 2013 season. Each value represents three replications (total 4 points per plot) from field isolated, light acclimated plants on the youngest fully expanded leaf at vegetative stages and the ear leaf at reproductive stages. Note: Only 2 reps were completed for R2 and R3 stage measurements

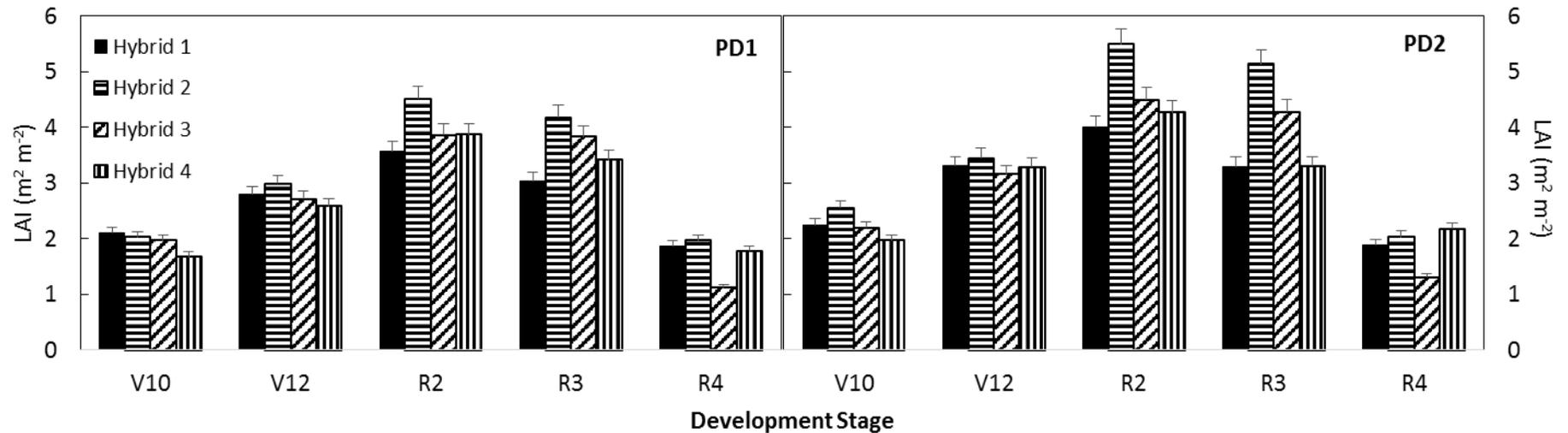


Figure 3.6 - Factorial for Hybrid x PD for Leaf Area Index (LAI) ($\text{m}^2 \text{m}^{-2}$) for all maize hybrids (Hybrid 1 = AQUAmax™ P1151, Hybrid 2 = P1162, Hybrid 3 = AQUAmax™ P1498, Hybrid 4 = 33D49) grown at two plant densities (PD1=78,000, PD2=99,000 pl ha^{-1}) and four N rates (Nr1 = 0, Nr2 = 134, Nr3 = 202, and Nr4 = 269 kg ha^{-1}) at various growth stages throughout the 2013 season. Mean values represent the average of 3 replications of 3 measurements per plot

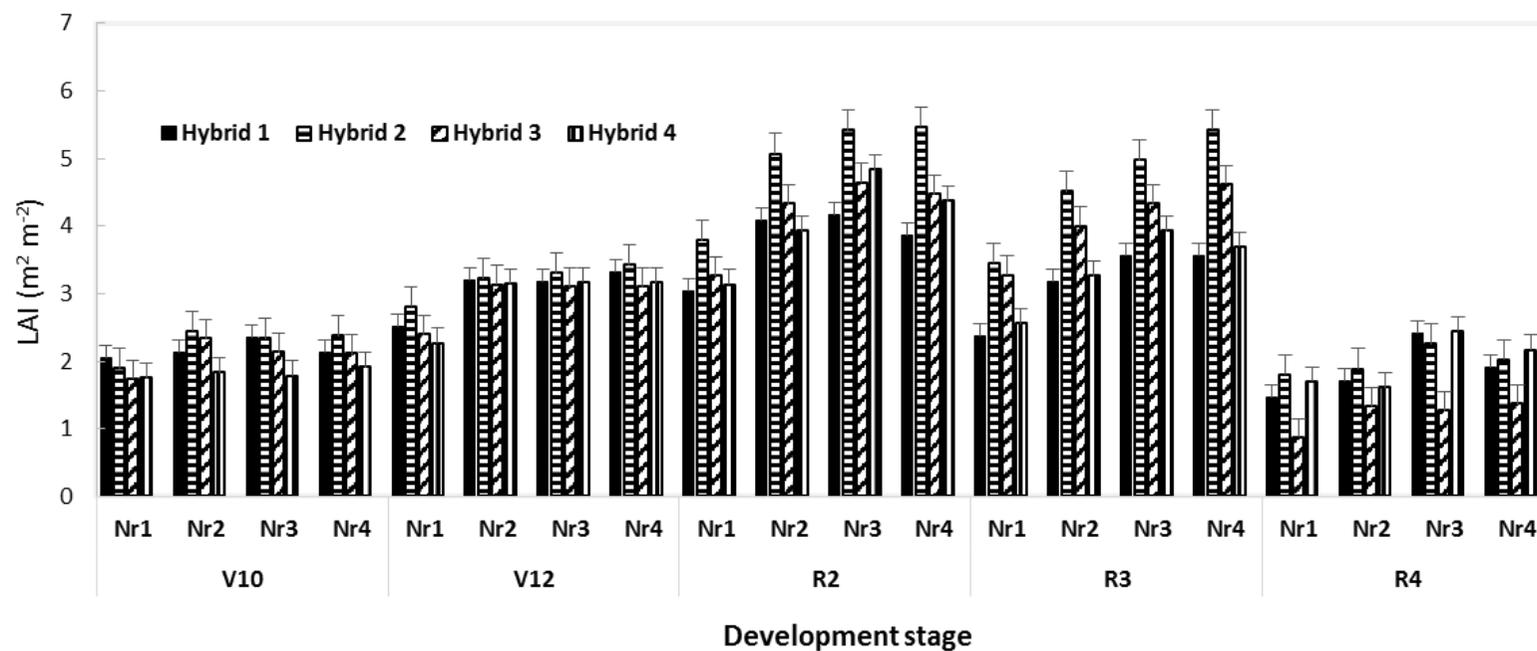


Figure 3.7 - Factorial for Hybrid x Nr for Leaf Area Index (LAI) ($\text{m}^2 \text{m}^{-2}$) for all maize hybrids (Hybrid 1 = AQUAmaxTM P1151, Hybrid 2 = P1162, Hybrid 3 = AQUAmaxTM P1498, Hybrid 4 = 33D49) grown at two plant densities (PD1=78,000, PD2=99,000 pl ha⁻¹) and four N rates (Nr1 = 0, Nr2 = 134, Nr3 = 202, and Nr4 = 269 kg ha⁻¹) at various growth stages throughout the 2013 season. Mean values represent the average of 3 replications of 3 measurements per plot

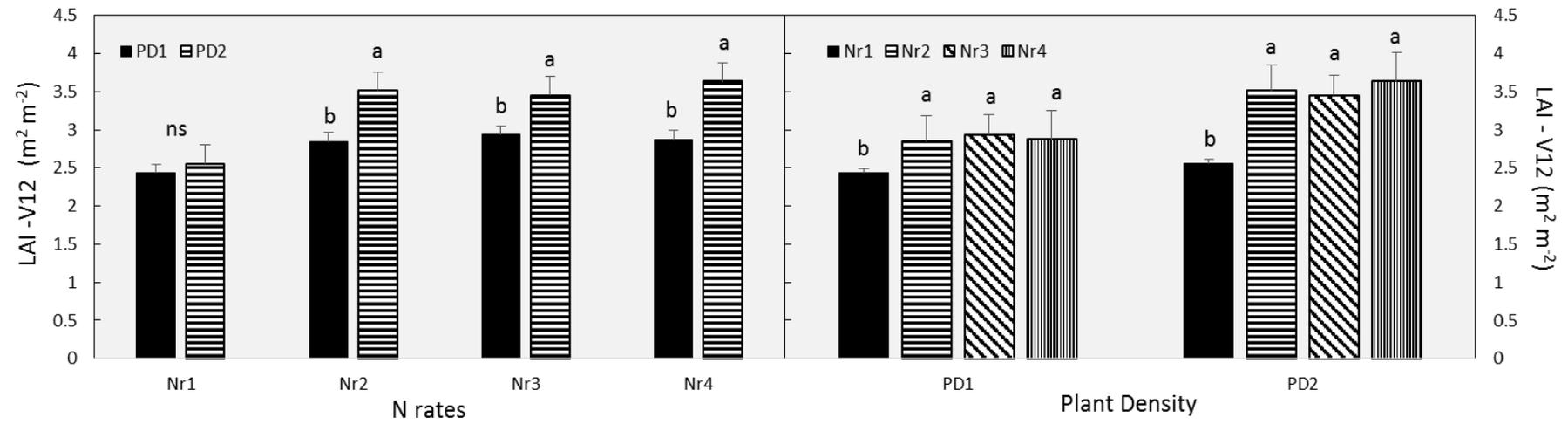


Figure 3.8 - Mean separation test for Leaf Area Index (LAI) ($\text{m}^2 \text{m}^{-2}$) for the factorial PD x Nr at V12 stage, for all maize hybrids (Hybrid 1 = AQUAmax™ P1151, Hybrid 2 = P1162, Hybrid 3 = AQUAmax™ P1498, Hybrid 4 = 33D49) grown at two plant densities (PD1=78,000, PD2=99,000 pl ha^{-1}) and four N rates (Nr1 = 0, Nr2 = 134, Nr3 = 202, and Nr4 = 269 kg ha^{-1}) in 2013

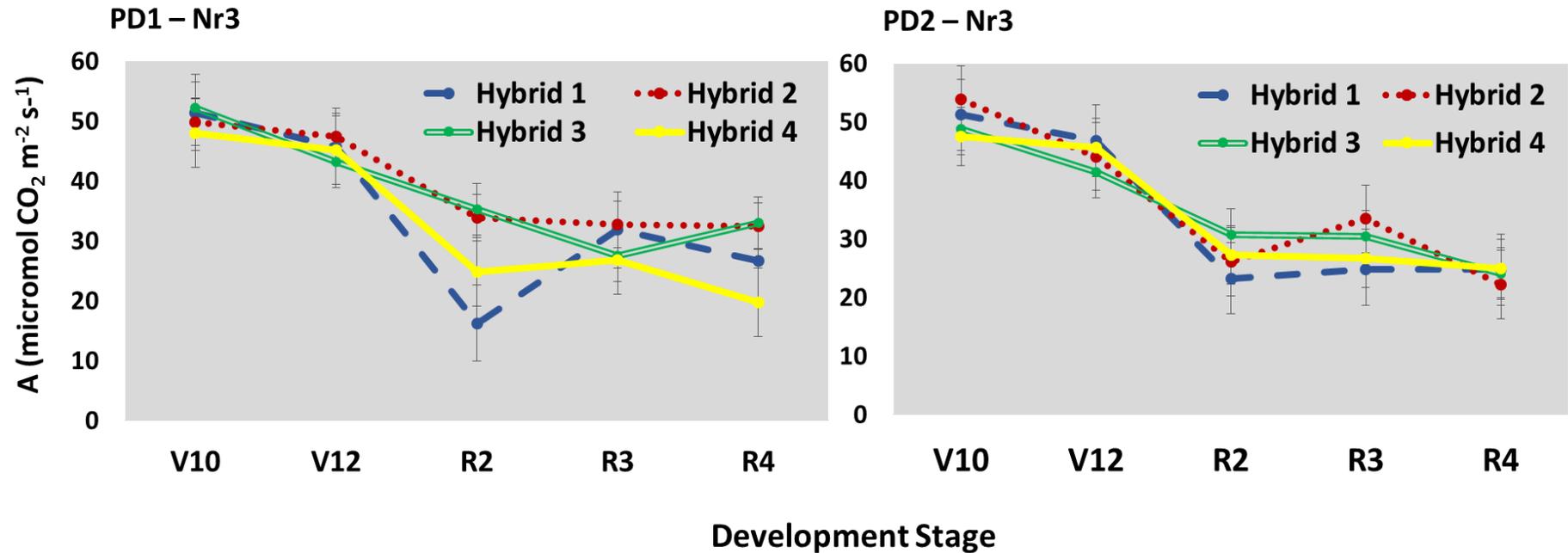


Figure 3.9 - A – Photosynthesis rates ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) using Licor 6400XT (LI-COR, Lincoln, NE) for all hybrids (Hybrid 1 = AQUAmaxTM P1151, Hybrid 2 = P1162, Hybrid 3 = AQUAmaxTM P1498, Hybrid 4 = 33D49) at both densities (PD1 = 78,000 pl ha⁻¹, PD2 = 99,000 pl ha⁻¹) and at only one N rate (Nr3 = 202 kg N ha⁻¹) at various growth stages throughout the 2013 season. Each value represents three replications (total 4 points per plot) from field isolated, light acclimated plants on the youngest fully expanded leaf at vegetative stages and the ear leaf at reproductive stages. Note: Only 2 reps were completed for R2 and R3 stage measurements

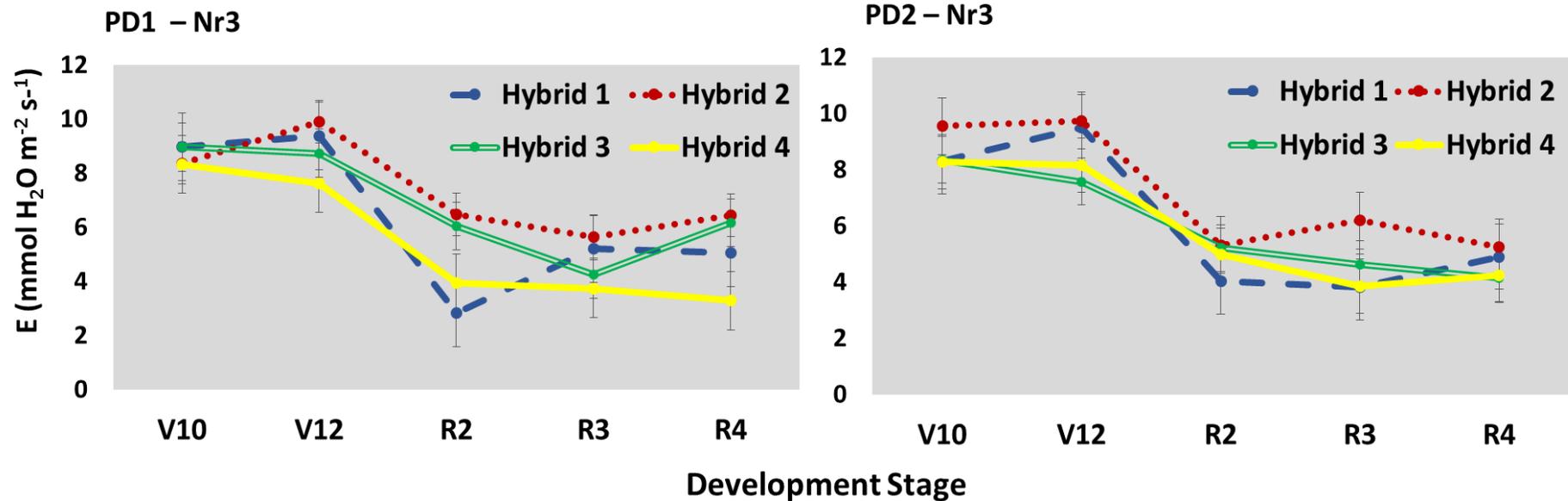


Figure 3.10 - E – Leaf transpiration rates ($\mu\text{mol H}_2\text{O m}^{-2} \text{s}^{-1}$) using Licor 6400XT (LI-COR, Lincoln, NE) for all hybrids (Hybrid 1 = AQUAmax™ P1151, Hybrid 2 = P1162, Hybrid 3 = AQUAmax™ P1498, Hybrid 4 = 33D49) at both densities (PD1 = 78,000 pl ha^{-1} , PD2 = 99,000 pl ha^{-1}) and at only one N rate (Nr3 = 202 kg N ha^{-1}) at various growth stages throughout the 2013 season. Each value represents three replications (total 4 points per plot) from field isolated, light acclimated plants on the youngest fully expanded leaf at vegetative stages and the ear leaf at reproductive stages. Note: Only 2 reps were completed for R2 and R3 stage measurements

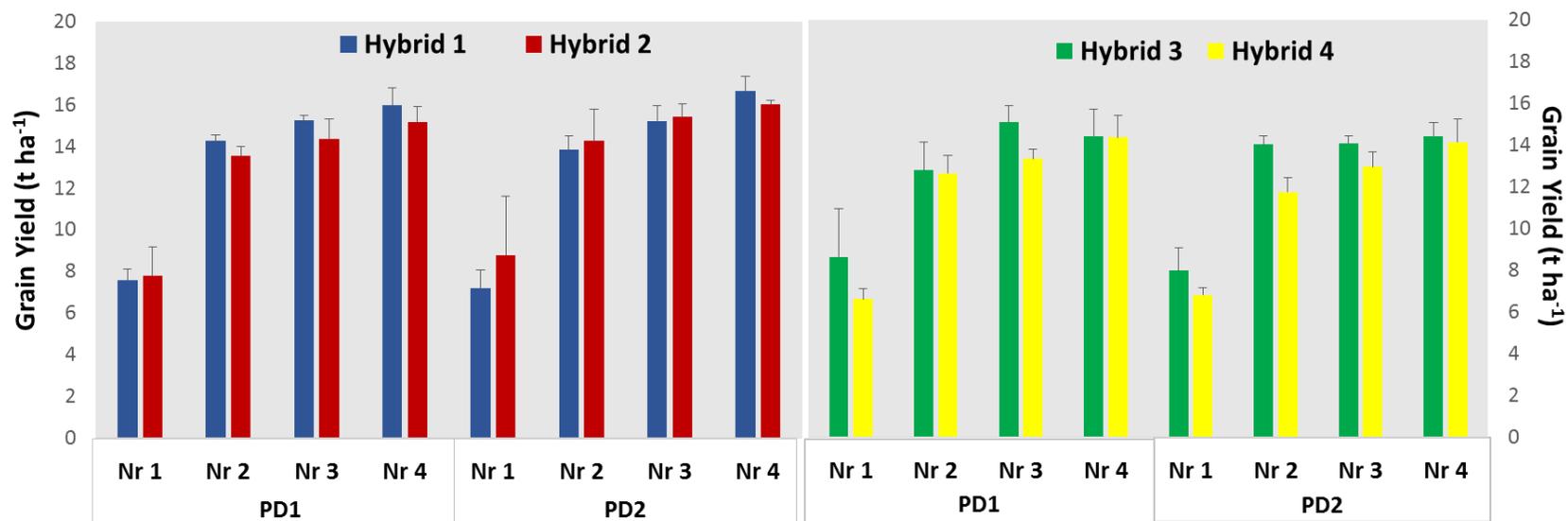


Figure 3.11 - Grain yields (t ha^{-1}) at physiological maturity for all maize hybrids (Hybrid 1 = AQUAmaxTM P1151, Hybrid 2 = P1162, Hybrid 3 = AQUAmaxTM P1498, Hybrid 4 = 33D49, and Hybrid 5 = P1184) grown at two plant densities (PD1 = 78,000, PD2 = 99,000 pl ha^{-1}), and four N rates (Nr1 = 0, Nr2 = 134, Nr3 = 202, and Nr4 = 269 kg ha^{-1}) in 2013

References

- BANZIGER, M.; EDMEADES, G.O.; LAFITTE, H.R. Physiological mechanisms contributing to the increased N stress tolerance of tropical maize selected for drought tolerance. **Field Crops Research**, Amsterdam, v. 75, p. 223-233, 2002.
- BANZIGER, M.; EDMEADES, G.O.; BECK, D.; BELLON, M. **Breeding for drought land nitrogen stress tolerance in maize: from theory to practice**. Mexico: CIMMYT, 2000. 68 p.
- BENEŠOVA, M.; HOLA, D.; FISCHER, L.; JEDELSKY, P.L.; HNILIČKA, F.; WILHELMOVA, N. The physiology and proteomics of drought tolerance in maize: Early stomatal closure as a cause of lower tolerance to short-term dehydration? **PLoS ONE**, San Francisco, v. 7, n. 6, e38017, 2012.
- BERGAMASCHI, H.; DALMAGO, G.A.; BERGONCI, J.I.; BIANCHI, C.A.M.; MÜLLER, A.G.; COMIRAN, F.; HECKLER, B.M.M. Distribuição hídrica no período crítico do milho e produção de grãos. **Pesquisa Agropecuária Brasileira**, Brasília, v. 39, p. 831-839, 2004.
- BERGAMASCHI, H.; DALMAGO, G.A.; COMIRAN, F.; BERGONCI, J.I.; MÜLLER, A.G.; FRANÇA, S.; SANTOS, A.O.; RADIN, B.; BIANCHI, C.M.M.; PEREIRA, P.G. Déficit hídrico e produtividade da cultura do milho. **Pesquisa Agropecuária Brasileira**, Brasília, v. 41, n. 2, p. 243-249, 2006.
- BERRY, J.A.; BJÖRKMAN, O. Photosynthetic response and adaptation to temperature in higher plants. **Annual Review of Plant Physiology and Plant Molecular Biology**, Palo Alto, v. 31, p. 491–543, 1980.
- BLUM, A.; ARKIN, G.F. Sorghum root growth and water use as affected by water supply and growth duration. **Field Crops Research**, Amsterdam, v. 9, p. 131-142, 1984.
- BOOMSMA, C.R.; SANTINI, J.B.; TOLLENAAR, M.; VYN, T.J. Maize morpho-physiological responses to intense crowding and low nitrogen availability: An analysis and review. **Agronomy Journal**, Madison, v. 101, p. 1426-1452, 2009.
- BRUCE, W.B.; EDMEADES G.O.; BARKER T.C. Molecular and physiological approaches to maize improvement for drought tolerance. **Journal of Experimental Botany**, Oxford, v. 53, p 13– 25, 2002.
- BUNCE, J.A. Leaf transpiration efficiency of some drought-resistant maize lines. **Crop Science**, Madison, v. 50, p. 1409–1413, 2010.
- _____. Leaf transpiration efficiency of sweet corn varieties from three eras of breeding. **Crop Science**, Madison, v. 51, p 793–799, 2011.
- CAMPOS, H.; COOPER, M.; EDMEADES, G.O.; LÖFFLER, C.; SCHUSSLER, J.R.; IBANEZ, M. Changes in drought tolerance in maize associated with fifty years of breeding for yield in the U.S. Corn Belt. **Maydica**, Rome, v. 51, p 369–381, 2006.

CIAMPITTI, I.A.; VYN, T.J. A comprehensive study of plant density consequences on nitrogen uptake dynamics of maize plants from vegetative to reproductive stages. **Field Crops Research**, Amsterdam, v. 121, p. 2–18, 2001.

CIAMPITTI, I.A.; MURRELL, S.T.; CAMBERATO, J.J.; TUINSTRAN, M.; XIA, Y.; FRIEDEMANN, P.; VYN, T.J. Physiological dynamics of maize nitrogen uptake and partitioning in response to plant density and nitrogen stress factors: II. Reproductive phase. **Crop Science**, Madison, v. 53, n. 6, p. 2588-2602, 2013.

COOPER, M.; GHO, C.; LEAFGREN, R.; TANG, T.; MESSINA, C. Breeding drought-tolerant maize hybrids for the US corn-belt: discovery to product. **Journal of Experimental Botany**, Oxford, v. 65, n. 21, p. 6191-204, 2014.

COSTA, J.R.; PINHO, J.L.N.; BEZERRA, M.L.; AQUINO, B.F.; CAVALCANTE JR, A.T. Variáveis hídricas e morfológicas em cultivares de milho (*Zea mays*, L.) submetidas ao estresse hídrico em dois estádios fenológicos da cultura. In: CONGRESSO BRASILEIRO DE FISILOGIA VEGETAL, 8., 2001, Ilhéus. **Resumos...** Ilhéus: Sociedade Brasileira de Fisiologia Vegeta, 2001. p. 20.

DEMIREVSKA, K.; ZASHEVA, D.; DIMITROV, R.; SIMOVA-STOILOVA, L.; STAMENOVA, M.; FELLER, U. Drought stress effects on Rubisco in wheat: changes in the Rubisco large subunit. **Acta Physiology Plantarum**, Heidelberg, v. 31, p. 1129- 1138, 2009.

DING, L.; WANG, K.J.; JIANG, G.M.; BISWAS, D.K.; Xu, H.; LI, L.F.; LI, Y.H. Effects of nitrogen deficiency on photosynthetic traits of maize hybrids released in different years. **Annals of Botany**, Oxford, v. 96, p. 925-930, 2005.

DWYER, L. M.; TOLLENAAR, M.; HOUWING, L. A nondestructive method to monitor leaf greenness in corn. **Canadian Journal of Plant Science**, Ottawa, v. 71, p. 505-509, 1991.

DWYER, L.M.; ANDERSON, A.M.; STEWART, D.W.; MA, B.L., TOLLENAAR, M. Changes in maize hybrid photosynthetic response to leaf nitrogen, from preanthesis to grain fill. **Agronomy Journal**, Madison, v. 87, p. 1221–1225, 1995.

EARL, H. J.; TOLLENAAR, M. Maize leaf absorptance of photosynthetically active radiation and its estimation using a chlorophyll meter. **Crop Science**, Madison, v. 37, n. 2, p. 436-440, 1997.

EARL, H.J.; TOLLENAAR, M. Using chlorophyll fluorometry to compare photosynthetic performance of commercial maize (*Zea mays* L.) hybrids in the field. **Field Crops Research**, Amsterdam, v. 61, p. 201-210, 1999.

EAMUS, D.; TAYLOR, D.T.; MACINNIS-NG, C.M.O.; SHANAHAN, S.; DE SILVA, L. Comparing model predictions and experimental data for the response of stomatal conductance and guard cell turgor to manipulations of cuticular conductance, leaf-to-air vapour pressure difference and temperature: feedback mechanisms are able to account for all observations. **Plant, Cell and Environment**, Oxford, v. 31, p. 269-277, 2008.

ECHARTE, L.; ROTHSTEIN, S.; TOLLENAAR, M. The response of leaf photosynthesis and dry matter accumulation to nitrogen supply in an older and a newer maize hybrid. **Crop Science**, Madison, v. 48, p. 656-665, 2008.

EDWARDS, G.; WALKER, D. **C3, C4: mechanisms and cellular and environmental regulation of photosynthesis**. Berkeley; Davis: University of California Press, 1983.

HATTERSLEY, P.W. Characterization of C4 type leaf anatomy in grasses (*Poaceae*). Mesophyll: bundles sheath area ratios. **Annals of Botany**, Oxford, v. 53, p. 163-179, 1984.

JALEEL, C.A.; PARAMASIVAM, M.; WAHID, A.; FAROOQ, M.; AL-JUBURI, H.J.; SOMASUNDARAM, F.; PANNEERSELVAM, R. Drought stress in plants: A review on morphological characteristics and pigment compositions. **International Journal of Agriculture and Biology**, Faisalabad, v. 11, p. 100-105, 2009.

KIM, S.H.; SICHER, R.C.; BAE, H.; GITZ, D.C.; BAKER, J.T.; TIMLIN, D.J.; REDDY, V.R. Canopy photosynthesis, evapotranspiration, leaf nitrogen, and transcription profiles of maize in response to CO₂ enrichment. **Global Change Biology**, Chichester, v. 12, p. 588-600, 2006.

LEAKEY, A.D.B.; URIBELARREA, M.; AINSWORTH, E.A.; NAIDU, S.L.; ROGERS, A.; ORT, D.R.; LONG, S.P. Photosynthesis, productivity, and yield of maize are not affected by open-air elevation of CO₂ concentration in the absence of drought. **Plant Physiology**. Rockville, v. 140, p. 779-790, 2006.

LI, Y.; SPERRY, J. S.; SHAO, M. Hydraulic conductance and vulnerability to cavitation in corn (*Zea mays* L.) hybrids of differing drought resistance. **Environmental and Experimental Botany**, Oxford, v. 66, p. 341-346, 2009.

MA, B.L.; DWYER, L.M. Nitrogen up take and use of two contrasting maize hybrids differing in leaf senescence. **Plant and soil**, Dordrecht, v. 199, p. 283-291, 1998.

MARKELZ, R.J.C.; STRELLNER, R.S.; LEAKEY, A.D.B. Impairment of C4 photosynthesis by drought is exacerbated by limiting nitrogen and ameliorated by elevated [CO₂] in maize. **Journal of Experimental Botany**, Oxford, v. 62, p. 3235-3246, 2011.

MONNEVEUX, P.; SANCHEZ, C.; BECK, D.; EDMEADES, G.O. Drought tolerance improvement in tropical maize source populations: evidence of progress. **Crop Science**, Madison, v. 46, p. 180-191, 2006.

PIONEER. **Optimum AQUAmax™ products from DuPont Pioneer**. Disponível em: <https://www.pioneer.com/CMRoot/Pioneer/US/products/seed_trait_technology/see_the_difference/AQUAmax_Product_Offerings.pdf>. Acesso em: 15 dez. 2013.

_____. Disponível em: <<http://www.pioneersementes.com.br/Media Center/Pages/Detaildo-Artigo.aspx?p=165>>. Acesso em: 08 jul. 2014.

PRANGE, R.K.; McRAE, K.B.; MIDMORE, D.J., DENG, R. Reduction in potato growth at high-temperature - role of photosynthesis and dark respiration. **American Potato Journal**, New York, v. 67, p. 357-369, 1990.

ROTH, J.; CIAMPITTI, I.A.; VYN, T.J. Physiological evaluations of recent drought-tolerant maize hybrids at varying stress levels. **Agronomy Journal**, Madison, v. 105, p. 1129-1141, 2013.

SADOK, W.; SINCLAIR, T.R. Genetic variability of transpiration response of soybean (*Glycine max* (L.) Merr.) shoots to leaf hydraulic conductance inhibitor AgNO₃. **Crop Science**, Madison, v. 50, p. 1423-1430, 2010.

SANCHEZ, R.A.; HALL, A.J.; TRAPANI N.; COHEN DE HUNAU, R. Effects of water stress on chlorophyll content, nitrogen level and photosynthesis of leaves of two maize genotypes. **Photosynthesis Research**, Dordrecht, v. 4, p. 35-47, 1983.

SANGOI, L. Understanding plant density effects on maize growth and development: an important issue to maximize grain yield. **Ciência Rural**, Santa Maria, v. 31, n. 1, p. 159-168, 2000.

SAS INSTITUTE. **SAS/STAT 9.1**: users guide. Cary, 2004. 824 p.

SCHITTENHELM, S. Chemical composition and methane yield of maize hybrids with contrasting maturity. **European Journal of Agronomy**, Córdoba, v. 29, n. 2, p. 72-79, 2008.

_____. Effect of drought stress on yield and quality of maize/sunflower and maize/sorghum intercrops for biogas production. **Journal of Agronomy and Crop Science**, Berlin, v. 196, n. 4, p. 253-261, 2010.

SHAO, H.; CHU, L.; JALEEL, C. A.; ZHAO, C. Water-deficit stress induced anatomical changes in higher plants. **Comptes Rendus Biologies**, Paris, v. 331, p. 215-225, 2008.

SINCLAIR, T.R.; ZWIENIECKI, M.A.; HOLBROOK, N.M. Low leaf hydraulic conductance associated with drought tolerance in soybean. **Physiology Plantarum**, Oxford, v. 132, p. 446-451, 2008.

SPANNO, G.; DI FONZO, N.; PERROTTA, C.; PLATANI, C.; RONGA, G. Physiological characterization of 'stay green' mutants in durum wheat. **Journal of Experimental Botany**, London, v. 54, p. 1415-1420, 2003.

TOLLENAAR, M.; DAYNARD, T.B. Leaf senescence in short-season maize hybrids. **Canadian Journal of Science**, Ottawa, v. 58, p. 869-875, 1978.

TOLLENAAR, M.; LEE, E.A. Strategies for enhancing grain yield in maize. **Plant Breeding Reviews**, Hoboken, v. 34, p. 37-81, 2011.

TURNER, L.B. The effect of water stress on the vegetative growth of white clover (*Trifolium repens* L.), comparative of long-term water deficit and short-term developing water stress. **Journal of Experimental Botany**, Oxford, v. 42, p. 311-316, 1991.

TURNER, N.C.; OTOOLE, J.C.; CRUZ, R.T.; YAMBAO, E.B.; AHMAD, S.; NAMUCO, O.S.; DINGKHUM, M. Response of seven diverse rice cultivars to water deficit. II. Osmotic adjustment, leaf elasticity, leaf expansion, leaf death, stomatal conductance and photosynthesis. **Field Crops Research**, Amsterdam, v. 13, p. 273-286, 1986.

UHART, S. A.; ANDRADE, F. H. Nitrogen deficiency in maize. I. Effects on crops growth, development, dry matter partitioning, and kernel set. **Crop Science**, Madison, v. 35, p. 1376-1383, 1995.

WEILAND, P. Biomass digestion in agriculture: a successful pathway for the energy production and waste treatment in Germany. **Engineering Life Science**, Dresden, v. 6, n. 3, p. 302-309, 2006.

WELCKER, C.; BOUSSUGE, B.; BENCIVENNI, C.; RIBAUT, M.; TARDIEU, F. Are source and sink strengths genetically linked in maize plants subjected to water deficit: a QTL study of the responses of leaf growth and of anthesis-silking Interval to water deficit. **Journal of Experimental Botany**, London, v. 58, p. 339-349, 2007.

WOLFE, D.W.; HENDERSON, D.W.; HSIAO, T.C.; ALVINO, A. Interactive water and nitrogen effects on senescence of maize. 1. Leaf area duration, nitrogen distribution, and yield. **Agronomy Journal**, Madison, v. 80, p. 859-864, 1988.

WU, Y.; HUANG, M.; WARRINGTON, D.N. Growth and transpiration of maize and winter wheat in response to water deficits in pots and plots. **Environmental and Experimental Botany**, Paris, v. 71, n. 1, p. 65-71, 2011.

XIA, Y. **Photosynthesis-related physiological responses of field-grown maize to plant density and nitrogen stress during vegetative and reproductive stages**. 2012. 181 p. Dissertation. (Ph.D. in Agronomy) - Purdue University, West Lafayette, 2012.

4 MAIZE RESPONSE TO NITROGEN APPLICATION TIMING AND CORRELATION AMONG LEAF VARIABLES WITH GRAIN YIELD AND COMPONENTES

Abstract

The knowledge about the effective influence of the factors that determine the performance of the plant can contribute decisively to minimize the stress caused by nitrogen deficiency. Thus, it is extremely important to obtain more reviews related to the correlation between the real content of chlorophyll and real carotenoids with the values obtained by chlorophyll (SPAD) in the early development stages of the maize. The development of research in relation to the maize crop response to the nitrogen fertilization at different development stages is as well as important. The primary objective of this study was to investigate the responses of maize to the nitrogen application, urea fertilizer (^{15}N), in sidedress at different development stages. The secondary objective was verifying the correlation between chlorophylls and carotenoids with SPAD index and these with total biomass (BM), harvest index (HI), grain yield (GY) and grain N content in response to the nitrogen side-dress at different development stages. The hybrid used in this study was the 30F35HR. The nitrogen fertilization was carried out in plots, with the application of 30 kg ha^{-1} of N at planting and 140 kg ha^{-1} N as side-dress at vegetative stages V4, V6, V8, V10 and V12, without incorporation into the soil, and control treatment consisted of non-nitrogen side-dress application was also utilized. The 2011/2012 season presented higher precipitation than 2012/2013. Maize crop responded similarly for GY to the nitrogen application in sidedress both seasons, however the nitrogen application in the early stages caused higher values for leaf variables, leaf pigments and SPAD. Higher amount of nitrogen in all parts of the plants was observed in the 2011/2012 season than in 2012/2013, influenced by the adequate weather conditions at the nitrogen application moment. Grain N content from ^{15}N fertilizer and N uptake and efficiency were greater for early N applications. SPAD values correlated positively with most pigment variables at V16 in both seasons, thus proving that SPAD was an efficient instrument of indirect evaluation of chlorophylls and carotenoids in maize leaves at early stages. Chlorophyll b at V16 was positively correlated ($P < 0.05$) with grain N content, GY, and BM, and total chlorophyll at V16 was positively correlated with GY and grain N content. However the chlorophylls a and total, evaluated at V14, were negatively correlated with GY. So, measurement of real chlorophyll and carotenoid pigment contents should be done after V14 stage when studies aim to evaluate crop nutritional conditions and prescribe future grain production practices.

Keywords: Chlorophylls; Carotenoids; SPAD; Early growth stages

4.1 Introduction

The appropriate mineral nutrition is among the factors which have the greatest influence on the productivity increase of crops, and nitrogen (N) is the most essential required nutrient, especially for the maize, which is one of the crops that respond to high increases in productivity in response to N fertilization. The non-availability of N in the soil causes several

problems to crops such as: reduced leaf area, decrease in photosynthesis, developmental delays and reduced yield. On the other hand, excessive application of N to the soil implies in higher production costs and can cause environmental problems such as contamination of the water, contributing to the increase of global warming due to the formation of nitrous oxide.

Maize nitrogen requirements vary considerably in different plant development stages (ARNON, 1975). Although it is known that this crop requires about 20 kg ha⁻¹ of N for each ton of produced grain (FANCELLI, 2000; SOUSA; LOBATO, 2004) there is still some controversy about the ideal time for nitrogen application to this crop. Some authors state that the ideal time is to make the application at seeding or close to this time, but others report that it is ideal to apply at the later stages, thus avoiding losses by leaching and volatilization and increasing the efficiency of absorption and the use of the nitrogen fertilizer (COELHO, 1987; NEPTUNE; CAMPANELLI, 1980; CANTARELLA, 1993; SÁ, 1996; DA ROS et al., 1999; PAULETTI; COSTA, 2000; CERETTA et al., 2000; BASSO; CERETTA, 2000).

Great advances have been made to improve the nitrogen use efficiency (NUE), which is defined as the ratio of dry matter production unit by unit applied N. The ways of how to assess the nutritional status of the plants are among the evaluation factors of plant response to management practices. The use of indirect measurements to determine the nutritional status of plants has been the object of research for many crops. Research studies have shown that, for some crops, the concentration of chlorophyll or the greening of leaves is positively correlated with leaf N concentrations, because 70% of N contained in the leaves is in the chloroplasts, participating in the synthesis and the structure of chlorophyll molecules (MARENCO; LOPES, 2005). For this reason, the content of chlorophyll in the late vegetative stage has been related to the N nutritional status of various crops (ARGENTA et al., 2001).

The traditional methods used to determine the amount of chlorophyll in the leaf require the destruction of tissue samples and much work in the extraction and quantification processes. The development of portable chlorophyll meter (SPAD), which allows instantaneous measurements of the amount corresponding to its content in the leaf without destroying it, is an alternative to estimate the relative content of these pigments in the leaf (DWYER; TOLLENAAR; HOUWING, 1991; ARGENTA et al., 2001). There is a strong positive relationship between the SPAD values with the N concentration in the leaves of the plants, being more evident in the later growth stages (ARGENTA et al., 2001) and there is high correlation with chlorophyll content (DWYER; TOLLENAAR; HOUWING, 1991; CIAMPITTI et al., 2012).

The knowledge about the effective influence of the factors that determine the performance of the plant can contribute decisively to minimize the stress caused by nitrogen deficiency. Thus, it is extremely important to obtain more reviews related to the correlation between the real content of chlorophyll and real carotenoids with the values obtained by chlorophyll (SPAD) in the early development stages of the maize. The development of research in relation to the maize crop response to the nitrogen fertilization at different development stages is as well as important. Through these assessments it is possible to have a greater knowledge about the plant relationship with the environment in which it is grown and may have increases in the grain yield.

The primary objective of this study was to investigate the responses of maize due to nitrogen [urea fertilizer (^{15}N)] side-dress application at different development stages. The secondary objective was verifying the correlation between the chlorophylls and carotenoids with the SPAD index and these with, total biomass, harvest index (HI), grain yield (GY) and grain N content in response to the nitrogen side-dress in different development stages.

4.2. Materials and Methods

The study was carried out at Tanquinho Farm, and at *Centro de Energia Nuclear na Agricultura*, University of São Paulo (CENA / USP), in Piracicaba, SP. The experiment was conducted under field conditions during the seasons of 2011/2012 and 2012/2013, and was installed in December 2011 and completed in March 2012 and repeated from December 2012 to March 2013. The experimental design consisted of random blocks having four replications, managed under conventional tillage, with corn as the preceding crop. The hybrid used in this study was 30F35HR (PIONEER, 2014).

Prior to the implementation of the experiment, the chemical and physical characterization of the field soil was performed for the 0-20 cm layer. Nitrogen (N) fertilization was carried out in plots with urea, as source, corresponding to 30 kg N ha⁻¹ at planting and 140 kg N ha⁻¹ as sidedress, without incorporation. These N doses are recommended aiming for a high grain yield (10-12 t ha⁻¹) as in areas with high response to N application (CANTARELLA; RAIJ; CAMARGO, 1997). The treatments consisted of five urea fertilizer application times as sidedressings, corresponding to the vegetative stages V4, V6, V8, V10 and V12 as described by Ritchie, Hanway and Benson (2003). A control treatment consisted of non-nitrogen side-dress fertilization was also utilized.

Plots had 10 rows of corn with 10 m length, spaced 0.5 m apart, thus totaling an area of 50 m², corresponding to a population of 60,000 plants per ha. In each of the plots, mini

plots (0.5 m wide and 1.5 m long) were delimited for ^{15}N -urea application, at the same dose and time of the commercial urea application to the rest of the plot, .

Before the planting, for both seasons, weed control was performed with the herbicide glyphosate. Then the mechanical seeding was performed with approximately 3.3 seeds per meter density (already accounted for 10% more due to losses) in order to obtain a final stand of 60,000 plants per hectare. Along with seeding, planting fertilization was performed with the application of full dose of P and K, recommended by Cantarella, Rajj and Camargo (1997), considering the values measured by the soil chemical analysis and expecting a high productivity.

At the end of the crop cycle, grain yield (GY) and plant biomass (BM) were determined. The GY was determined by weighing the grains harvested with moisture correction to 13%. The BM was determined based on the wet weight of the residuals, followed by the correction of the previously determined moisture.

The plant material from the shoot was separated into stem, leaves + tassel + ear husks, cobs and grain. All the material was dried at 60 °C with forced air to a constant weight. Subsequently, the dried plant material was ground in a Wiley grinder, homogenized and subsampled. In all subsamples the nitrogen content (g kg^{-1}) was determined by Kjeldahl digestion - distillation and determining the sulfur content by the nitric perchloric digestion methodology followed by the turbidity determination.

The plants from the mini plots were collected and separated in grains, cobs, stalks and leaves, dried in an oven and finally ground for later determination (in grains) of total-N content and ^{15}N abundance in a mass spectrometer (BARRIE; PROSSER, 1996), only in the 2012/2013 season. Based on these values the grain nitrogen content derived from the fertilizer (GNCF) as well as the nitrogen fertilizer use and efficiency (NFUE) were calculated according to Gava et al. (2006).

4.2.1 Descriptions of the Analyzed Variables

4.2.1.1 Non-destructive Measures:

Indirect or SPAD chlorophyll content index (Soil Plant Analysis Development), was obtained by using the SPAD-502 chlorophyll meter (Minolta, Japan) (MINOLTA, 1989; PESTANA et al., 2001; MARKWELL; OSTERMAN; MITCHELL, 1995) on two uppermost leaves per plant, fully developed, before getting into senescence and being photosynthetically

active. Six samples per leaf were performed in 4 plants per treatment, carried out at V14 to V16 stages.

Plant height, evaluated on 4 plants per treatment at the flowering period (VT), variable obtained with a measuring tape (in centimeters) placed from soil surface to the highest insertion of the last uppermost leaves.

4.2.1.2 Destructive Measures:

Content of pigments in the leaves, evaluated in laboratory. The same leaves of SPAD evaluation were collected and taken to the laboratory for the analysis of chlorophyll a, b, and total and carotenoids. Following the methodology adapted from Moran and Porath (1980), two leaves per plant were evaluated from a total of 4 plants per treatment at V14 and V16 stages.

4.2.2 Statistical Analysis

The data were tested regarding the normality of the errors, as well as the homogeneity of variances, and then subjected to analysis of variance at 5% of significance. Having significant effects of the treatments by the F test, comparisons were performed using the t test, also at the level of 5% significance. The Pearson correlation test was also performed among the variables in order to verify the existence of positive correlations.

Table 4.1 - Soil analysis in both maize growing seasons, 2011/2012 and 2012/2013, (inorganic nitrogen [NO_3^- - N/NH_4^+ -N], soil pH, potassium content [K], and phosphorus Bray-P 1 [P]) in from 0 – 20 and 20 – 40 of the soil profile

Profile	pH	P	K	Ca	Mg	H+Al	Al	T	V	OM ¹	Silt	Clay
cm	(CaCl ₂)	mg dm ⁻³	----- mmol _c dm ⁻³ -----			-----		%	----- g kg ⁻¹ -----			
2011/12												
0–20	4.9	27	1.9	30	13	42	1	87	52	29	151	529
20–40	4.5	32	0.6	19	8	58	5	86	32	22	102	548
2012/13												
0–20	4.8	29	1.3	16	9	47	2	73	36	30	-	-
20–40	4.6	21	0.6	10	7	52	3	70	25	24	-	-

¹ = Organic matter

4.3 Result and Discussion

4.3.1 Growing Season and Phenology

The weather conditions during both whole growing seasons can be seen in Figure 1.2. The 2011/2012 season presented higher precipitation than 2012/2013, however in the 2012/2013 there was regular precipitation during the stages of N application (Figure 4.1).

4.3.2 Nitrogen uptake and partitioning, and Sulfur Uptake

In the 2011/2012 season (Table 4.2), treatments were significant for most variables, but not for cob N content. For leaf N content there was only difference between the control and all N applications, in all development stages. But for stem N content only the N application in V4 and V6 were different than the control ($V4 = V6 > \text{control}$). Grain N content and total plant N uptake were greater for V6 and V10 than the control. However the N application at V8 resulted in similar values in relation to the control for both variables. Nonetheless total plant sulfur uptake was similar for all treatments with nitrogen application. We observed a higher amount of nitrogen in all parts of the plants in the 2011/2012 season as compared to the 2012/2013 (Table 4.3). This was more consistent for total plant N uptake that presented mean value of 50 kg ha^{-1} of N more than the second season (2012/2013).

Maize responded differentially between seasons, this could have happened because after the stages that presented better variable means, a well distributed precipitation spell was observed (very close to the nitrogen application in side-dress), while in other stages there was a lack or small amount of precipitation that could have influenced the N uptake and partitioning, through losses by volatilization or percolation in the soil, hindering the hydrolysis of urea and its absorption. For urea application in dry soil conditions, 70% of the applied nitrogen remains on the soil in the hydrolyzed form (SHERLOCK; SMITH, 1987).

Fertilizer N in grain content (GNCF) and nitrogen fertilizer use and efficiency (NFUE) calculated from ^{15}N data, were greater in the early applications ($V4 = V6 > V8, V10, \text{ and } V12$) $p < 0.0001$. These variables presented lower values with the later N application, being the V12 application lowest one for GNCF, presenting a mean of 20, respectively, for both GNCF (kg ha^{-1}) and NFUE (%), lower the N application at V4 stage. This could have happened because the plants had a longer period to perform the process N of uptake from the soil, nevertheless the weather conditions had a greater influence on this. Coelho (1987) reports that the full implementation of N rates at planting provides higher maize biomass gain per kg of N than the gains from the sidedressing N.

Table 4.2 - Nitrogen partitioning (kg ha⁻¹ dry weight) into leaf, stem, cob, grain, and total plant components; and total plant sulfur uptake (kg ha⁻¹ dry weight) at physiological maturity, in response to the nitrogen application as side-dress at the V4, V6, V8, V10, and V12 stages in the 2011/2012 season

Treat	Leaves N (kg ha ⁻¹)	Stem N (kg ha ⁻¹)	Cob N (kg ha ⁻¹)	Grain N (kg ha ⁻¹)	Total N (kg ha ⁻¹)	Total S (kg ha ⁻¹)
Control	40.9 b	15.2 b	8.4	85.7 bc	150.2 c	8.9 c
V4	50.0 a	24.0 a	10.5	115.7 ab	200.3 ab	12.9 ab
V6	55.5 a	25.9 a	11.6	125.6 a	218.6 a	13.2 ab
V8	55.2 a	23.8 ab	8.7	78.9 c	166 .0 bc	11.3 b
V10	53.5 a	21.2 ab	10.9	131.7 a	217.4 a	13.5 a
V12	49.9 a	18.0 ab	11.7	110.5 abc	190.1 abc	12.3 ab
ANOVA						
Trat	*	*	ns	*	*	**
CV%	11.4	27.3	29.4	21.8	15.4	12.9

ns = not significant; *=P<0.05; **=P<0.01; ***=P<0.0001

Table 4.3 - Nitrogen partitioning (kg ha⁻¹ dry weight) into leaf, stem, cob, grain, and total plant components; Sulfur total plant uptake (kg ha⁻¹ dry weight); grain nitrogen content from the fertilizer (GNCF), and nitrogen fertilizer use and efficiency (NFUE) at physiological maturity, in response to the nitrogen application as sidedress at the V4, V6, V8, V10, and V12 stages in the 2012/2013 season

Treat.	Leaves N (kg ha ⁻¹)	Stem N (kg ha ⁻¹)	Cob N (kg ha ⁻¹)	Grain N (kg ha ⁻¹)	Total N (kg ha ⁻¹)	Total S (kg ha ⁻¹)	GNCF (kg ha ⁻¹)	NFUE %
Control	35.3	10.6 c	6.6	129.6	182.1	13.6 c	-	-
V4	45.5	15.1 ab	6.8	127.6	195.0	14.7 bc	46.1 a	48.9 a
V6	43.4	16.3 a	7.6	130.7	198.1	16.4 ab	49.7 a	51.8 a
V8	39.0	15.2 ab	8.3	121.9	184.4	15.5 abc	31.9 b	31.2 b
V10	39.3	15.8 ab	9.0	135.6	199.7	17.7 a	27.0 bc	26.7 bc
V12	41.8	13.4 bc	8.8	145.7	209.8	17.4 a	17.7 c	17.8 c
ANOVA								
Trat	ns	**	ns	ns	ns	*	***	***
CV%	13.7	12.8	15.5	7.7	7.8	11.3	20.56	19.9

ns = not significant; *=P<0.05; **=P<0.01; ***=P<0.0001

4.3.3 Grain yield, biomass and harvest index

In 2011/2012, total plant biomass was not influenced by treatments (Table 4.4), only cob weight had significant differences (p<0.05). Therefore in 2012/2013 (Table 4.5) there was only difference for HI, been the V12 application, which was better than most, but not better

than V8. França et al. (1994) reported that the splittings of N do not affect the efficiency of nitrogen fertilizer or use of N from the soil and the results obtained were similar when they applied up to 106 kg of N per ha in a single dose at the stage where the plant had 6 leaves or subdivided twice, half at the 6-leaf stage and the other half at the stage where the plant had 10 leaves. These authors also observed that most of the N in the plant was accumulated until flowering, reaching values of up to 93%. They concluded that the nitrogen sidedressing should be made after seeding until the early flowering, a period during which the rate of absorption is virtually linear. The N application efficiency prior to maize planting was studied by many authors (SA, 1996; PAULETTI; COSTA, 2000; CERETTA et al., 2000). All of them found little difference between the time of N application, but Ceretta et al. (2000) warned that the early application can compromise yield in years of high rainfall, in the early stage of crop development.

However, Jokela and Randall (1989) concluded that there was less response of maize to N when it was applied at the V2 stage than at the V8 stage. Maize starts to take up N rapidly at the middle vegetative growth period (V10) and the maximum rate of N uptake occur near to silking (HANWAY, 1963; SETTIMI; MARANVILLE., 1998). Hence, application of N at V8 - V10 stage should be one of the best ways of supplying N to convene this high demand.

Table 4.4 - Plant biomass (t ha⁻¹) of dry mass of leaf, stem, cob, grain, and total plant components; grain yield (GY); and grain harvest index (HI) at physiological maturity, in response to the nitrogen application as side-dress at the V4, V6, V8, V10, and V12 stages in the 2011/2012 season

Treat.	Leaves Weight (t ha⁻¹)	Stem Weight (t ha⁻¹)	Cob Weight (t ha⁻¹)	Total Weight (t ha⁻¹)	GY (t ha⁻¹)	HI %
Control	4.6	3.1	1.2 c	16.7	6.7	43.1
V4	5.3	4.1	1.5 ab	19.2	8.2	42.9
V6	5.3	4.2	1.6 ab	18.7	7.6	40.5
V8	4.8	3.6	1.3 bc	15.4	5.6	36.2
V10	5.1	3.8	1.6 a	20.0	9.4	47
V12	5.2	3.7	1.5 ab	18.9	8.4	44.1
ANOVA						
Trat	ns	ns	*	ns	ns	ns
CV%	9.5	17.7	12.6	16	15.5	11.6

ns = not significant; *=P<0.05; **=P<0.01; ***=P<0.0001

Table 4.5 - Plant biomass (t ha⁻¹) of dry mass of leaf, stem, cob, grain, and total plant components; grain yield (GY); and grain harvest index (HI) at physiological maturity, in response to the nitrogen application as side-dress at the V4, V6, V8, V10, and V12 stages in the 2012/2013 season

Treat.	Leaves Weight (t ha⁻¹)	Stem Weight (t ha⁻¹)	Cob Weight (t ha⁻¹)	Total Weight (t ha⁻¹)	GY (t ha⁻¹)	HI %
Control	4.6	3.2	1.5	19.9	11.1	53.1 b
V4	4.7	3.6	1.6	20.9	11.1	52.5 bc
V6	4.8	3.8	1.6	21.2	10.9	51.5 c
V8	4.4	3.2	1.6	20.0	10.1	53.9 ab
V10	4.6	3.4	1.6	20.9	10.6	53.4 b
V12	4.6	3.1	1.5	20.8	11.1	55.1 a
ANOVA						
Trat	ns	ns	ns	ns	ns	**
CV%	8.2	10.7	7.2	6.9	5.8	2

ns = not significant; *=P<0.05; **=P<0.01; ***=P<0.0001.

4.3.4 Leaf pigments content, SPAD, and plant height

Early N application triggered the highest value for most variables in both seasons. In 2011/2012 (Table 4.6), lower values than in 2012/2013 (Table 4.7) were observed for all variables. The differences were more expressive for the V14 stage than for V16 in most of variables. However, SPAD had significant differences for these two stages in both seasons, being greater from V4 to V10 than at the V14 stage and having only difference between N fertilized treatments with control for V16 in 2011/2012. However in 2012/2013 differences for SPAD were larger than in the first season. At the V14 sample stage, V6 was greater than V8, V10, V12, and control. Similar results for V16 were found, however V6 presented higher values for SPAD only in relation to V4, V12, and control. Plant high in 2011/2012 only was different than the control, and in 2012/2013 only V4 presented differences in relation to the control (V4 > Control). Chlorophyll a, b and total were also lower in the non-fertilized treatment than in treatments with N. According Campostrini (1998) and Fancelli and DouradoNeto (2004), chlorophyll a is the primary pigment composed of a magnesium ion in the centralized ring porphyrin. This ring contains four nitrogen atoms. The molecule has one hydrocarbon chain (hydrophobic), which has the maximum photon absorption in the blue region corresponding to the wavelengths 428 to 660 nm. The chlorophyll b is an accessory structure similar to the pigment chlorophyll, and in most plants, the ratio of chlorophyll a / b is around 3 to 1.14. Chlorophyll b has maximum absorption in the red region, from 452 and 641.8 nm.

4.3.5 Person correlations

SPAD, evaluated at V16, had a significant positive correlation with all pigments at both stages evaluated in 2011/2012 (Table 4.8), this variable correlated positively and significantly (both stages, V14 and V16) with the measured amount of total chlorophyll total, evaluated in laboratory conditions. The strongest SPAD V16 correlation related to pigments was with chlorophyll a in the V14 stage (0.61, $p < 0.05$). In the same way SPAD at V14 sample stage also presented strong correlation with other pigments, however this variable only had correlation with the variables evaluated at the same stage. The strongest correlation for SPAD, evaluated at V14, with pigments was with total chlorophyll (0.80, $p < 0.0001$). Nevertheless, in 2012/2013 (Table 4.9) there were significant correlations for SPAD with pigments only between SPAD/V14 with chlorophyll a and total (0.40, $p < 0.05$, for both) at the same stage.

Even with less correlation between SPAD and pigments in the second season, we can consider the efficiency of the SPAD measuring device to evaluate the actual amount of these pigments. These results are in accordance with other studies. According to Piekielek et al. (1995) and Dwyer et al., 1991, the indirect chlorophyll content in the leaf can be used to predict the nutritional N level in plants, because the correlation with the amount of pigment was positive in relation to N concentration. There is a strong positive relationship between the SPAD and N concentration in the leaves of the plants, although this is more evident in the later growth stages (ARGENTA et al., 2001), and there is a high correlation of SPAD with chlorophyll content (DWYER et al., 1991; CIAMPITTI et al., 2012). A significant positive correlation between SPAD/V16 and grain N content was found in the first season, but there was no significant correlation between SPAD with gain yield, harvest index, and grain N content in the second season (2012/2013). Chlorophyll b at V16 presented significant positive correlation with Grain N content (0.61, $p < 0.05$), GY (0.55, $p < 0.01$), and total biomass (0.53, $p < 0.05$), also the chlorophyll total at V16 presented a positive correlation with GY (0.50, $p < 0.05$) and grain N content (0.48, $p < 0.05$), however the chlorophylls a and total evaluated at V14 presented a negative significant correlation with GY. Thus, the measurement of pigment contents aiming to study nutritional crop conditions and predict grain production should be performed after the V14 stage. There is a strong positive relationship between the SPAD values with the N concentration in plant leaf, being more evident in the later growth stages (ARGENTA et al., 2001).

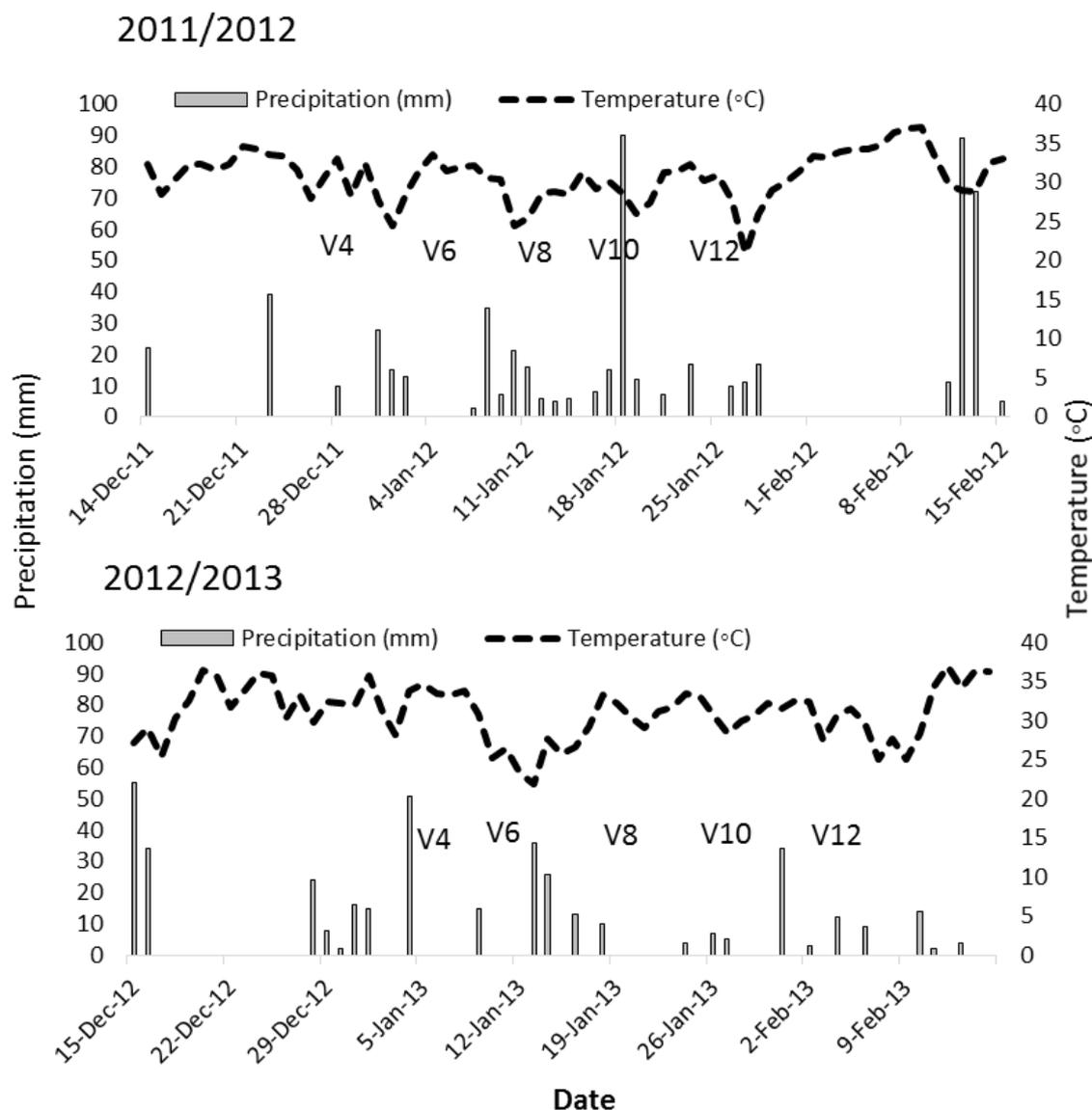


Figure 4.1 - Days of development stages (moment of nitrogen application in side-dress) and weather conditions (maximum air temperature and mean precipitation) for the 2011/2012 and 2012/2013 maize growing seasons at *Tanquinho* Farm, Piracicaba, Sao Paulo, Brazil

4.4 Conclusions

There was a similar response to the treatments for grain yield, harvest index and total plant biomass in both seasons. A higher amount of nitrogen was observed in all parts of the plants in the 2011/2012 season in relation to the 2012/2013, influenced by the weather conditions at the moment of nitrogen application.

Grain N content from the ^{15}N fertilizer (GNCF) and nitrogen fertilizer use and efficiency (NFUE) were greater in the early applications, at stages V4 and V6.

SPAD and leaf pigments were largely influenced by the evaluation stage. Evaluations made at V14 presented more differences in treatments than at V16, and the N application in the early stages caused higher values for most of the leaf variables (pigments and SPAD).

SPAD correlated positively and significantly with most pigment variables at V16, for both seasons, mainly in the first season that this variable was correlated with all chlorophylls and carotenoids, seeming to be an efficient instrument for the indirect evaluation of chlorophylls and carotenoids in maize leaves at early growth stages. Also SPAD in V16 sample stage had a positive correlation with grain nitrogen content and total plant biomass.

Chlorophyll b at V16 (2012/2013) presented a significant and positive correlation with grain N content, grain yield, and total biomass. Chlorophyll total at V16 also presented a positive correlation with grain yield and grain N content, however the chlorophylls a and total, evaluated at V14, presented a negative significant correlation with grain yield. So, measurement of real pigment contents aiming to study the nutritional crop conditions and predict grain production should be made after the V14 stage.

Table 4.6 - Leaf pigment contents (mg g⁻¹ fresh leaf mass): chlorophyll a (CA), chlorophyll b (CB), chlorophyll total (CT), carotenoids (Carot), SPAD, evaluated at V14 and V16 stages; and plant height (PH) at VT in response to the nitrogen application as side-dress at the V4, V6, V8, V10, and V12 stages, in the 2011/2012 season

Treat.	CA	CA	CB	CB	CT	CT	Carot	Carot	SPAD	SPAD	PH
	V14	V16	V14	V16	V14	V16	V14	V16	V14	V16	
	(mg g ⁻¹)			(cm)							
Control	0.770 c	0.77	0.25 c	0.24	1.02 c	1.01	0.16 c	0.17 b	41.1 c	38.0 b	259.1 b
V4	1.130 a	0.88	0.46 a	0.32	1.59 a	1.20	0.23 a	0.21 ab	55.2 a	51.5 a	290.2 a
V6	1.050 ab	0.9	0.37 ab	0.30	1.42 ab	1.19	0.22 ab	0.19 ab	51.0 a	51.2 a	283.2 a
V8	1.04 ab	0.92	0.39 ab	0.31	1.43 ab	1.23	0.22 ab	0.22 a	52.1 a	50.1 a	282.0 a
V10	0.947 abc	0.9	0.31 bc	0.28	1.26 bc	1.19	0.20 abc	0.21 ab	50.2 ab	50.0 a	287.3 a
V12	0.880 bc	0.83	0.29 bc	0.26	1.17 bc	1.09	0.19 bc	0.20 ab	45.0 bc	48.5 a	281.0 a
ANOVA											
Trat	*	ns	**	ns	*	ns	*	*	**	**	*
CV%	13.9	17.9	20.2	19.6	15.1	18.1	13.2	15.6	7.7	6.7	4.1

ns = not significant; *=P<0.05; **=P<0.01; ***=P<0.0001

Table 4.7 - Leaf pigment contents : chlorophyll a (CA), chlorophyll b (CB), chlorophyll total (CT), carotenoids (Carot), SPAD, evaluated at the V14 and V16 stages; and plant height (PH) at VT in response to the nitrogen application as side-dress at the V4, V6, V8, V10, and V12 stages, in the 2012/2013 season

Treat.	CA	CA	CB	CB	CT	CT	Carot	Carot	SPAD	SPAD	PH
	V14	V16	V14	V16	V14	V16	V14	V16	V14	V16	
	(mg g ⁻¹)			(cm)							
Control	1.26 c	1.18	0.40 b	0.35	1.66 b	1.53	0.29	0.21	50.6 e	45.9 c	226.2 b
V4	1.44 ab	0.98	0.61 a	0.38	2.04 a	1.30	0.32	0.12	58.3 ab	51.8 bc	242.5 a
V6	1.43 ab	1.19	0.52 ab	0.39	1.95 a	1.58	0.32	0.23	58.8 a	61.5 a	236.9 ab
V8	1.53 a	0.98	0.51 ab	0.28	2.04 a	1.26	0.31	0.12	56.3 bc	55.5 ab	237.5 ab
V10	1.43 ab	1.11	0.52 ab	0.31	1.94 a	1.42	0.35	0.14	56.0 c	54.8 ab	231.9 ab
V12	1.36 bc	1.22	0.55 ab	0.36	1.91 a	1.58	0.31	0.23	53.0 d	53.8 b	236.2 ab
ANOVA											
Trat	**	ns	*	ns	*	ns	ns	ns	***	**	ns
CV%	5	27.8	23.4	30.4	8.5	26.3	15.4	63.7	2.64	9.22	3.7

ns = not significant; *=P<0.05; **=P<0.01; ***=P<0.0001

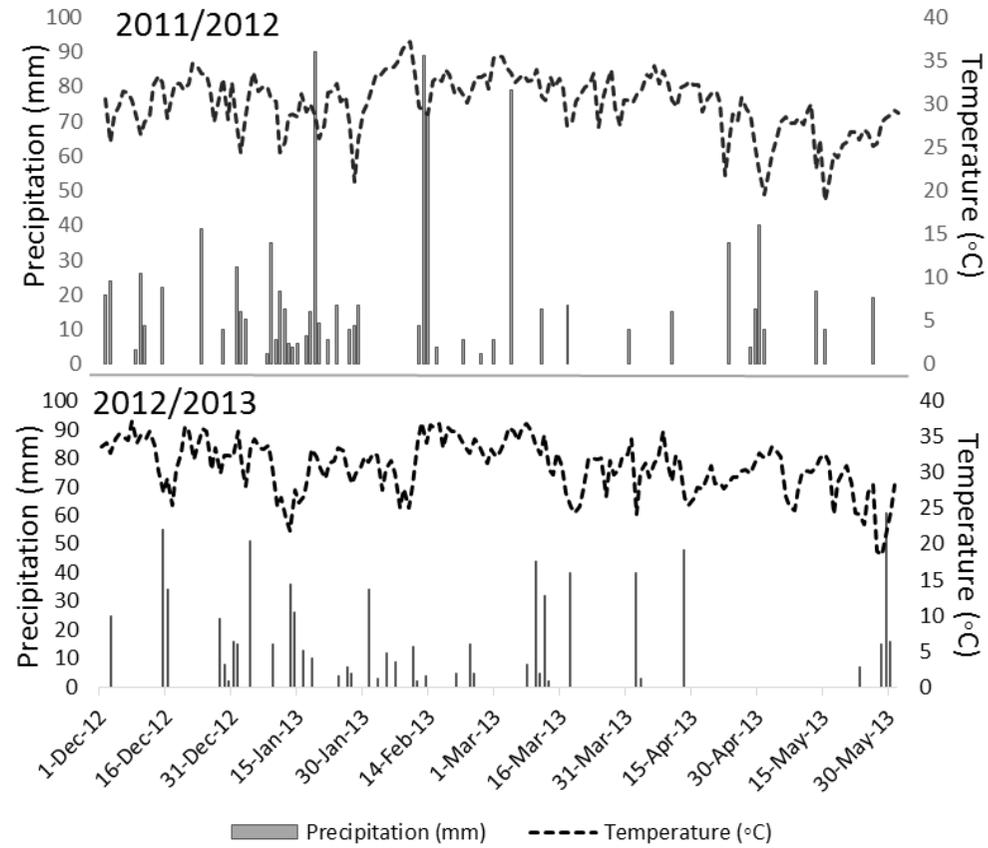


Figure 4.2 - Weather conditions (maximum air temperature and mean precipitation) for the 2011/2012 and 2012/2013 maize growing seasons at *Tanquinho* farm, Piracicaba, Sao Paulo, Brazil

Table 4.8 - Pearson correlation analysis for leaf pigment contents: chlorophyll a (CA), chlorophyll b (CB), chlorophyll total (CT), carotenoids (Carot), SPAD, evaluated at V14 and V16 stages; total plant biomass (BM), grain harvest index (HI), grain yield (GY), and grain nitrogen content (GNC) in response to the nitrogen application as side-dress at the V4, V6, V8, V10, and V12 stages, in the 2011/2012

	GNC	GY	HI	BM	SPAD16	SPAD14	Carot16	Carot14	CT16	CT14	CB16	CB14	CA16	CA14
CA14	0.06	0.2	-0.30	0.44	0.61**	0.76***	0.14	0.95***	0.20	0.99***	0.32	0.92***	0.14	1.00
CA16	0.13	0.17	-0.20	0.11	0.42*	0.23	0.08	0.07	0.99***	0.12	0.93***	0.08	1.00	
CB14	0.02	0.12	-0.22	0.05	0.52**	0.71***	0.90***	0.90***	0.12	0.97***	0.27	1.00		
CB16	0.1	0.14	-0.19	0.07	0.50*	0.40	0.24	0.12	0.97***	0.31	1.00			
CT14	0.05	0.15	-0.25	0.05	0.59**	0.80***	0.12	0.95***	0.20	1.00				
CT16	0.12	0.12	0.15	0.10	0.44*	0.23	0.84***	0.13	1.00					
Carot14	0.01	0.20	-0.30	0.04	0.60**	0.70***	0.13	1.00						
Carot16	0.01	0.05	-0.55	0.14	0.50**	0.4	1.00							
SPAD14	0.33	0.34	-0.05	0.34	0.75***	1.00								
SPAD16	0.50*	0.30	-0	0.45*	1.00									
BM	0.92***	0.70***	0.68	1.00										
HI	0.80***	0.50*	1.00											
GY	0.60**	1.00												
GNC	1.00													

*=P<0.05; **=P<0.01; ***=P<0.0001

Table 4.9 - Pearson correlation analysis for leaf pigment contents: chlorophyll a (CA), chlorophyll b (CB), chlorophyll total (CT), carotenoids (Carot), SPAD, evaluated at V14 and V16 stages; total plant biomass (BM), grain harvest index (HI), grain yield (GY), and grain nitrogen content (GNC) in response to the nitrogen application as side-dress at the V4, V6, V8, V10, and V12 stages, in the 2012/2013

	GNC	GY	HI	BM	SPAD16	SPAD14	Carot16	Carot14	CT16	CT14	CB16	CB14	CA16	CA14
CA14	-0.35	-0.61*	-0.35	-0.22	0.23	0.40*	-0.13	0.29	-0.18	0.83***	-0.22	0.41*	-0.14	1.00
CA16	0.40	0.38	0.22	0.24	0.12	-0.10	0.85***	-0.004	0.97***	0.23	0.59***	-0.25	1.00	
CB14	0.06	-0.22	-0.16	0.13	0.05	0.3	-0.12	0.34	-0.12	0.84***	-0.11	1.00		
CB16	0.61*	0.55**	-0.01	0.53*	0.31	0.12	0.075***	0.21	0.75***	-0.23	1.00			
CT14	-0.16	-0.50*	-0.30	-0.04	0.16	0.40*	-0.15	0.34	-0.24	1.00				
CT16	0.48*	0.50*	0.18	0.34	0.20	-0.05	0.90***	0.05	1.00					
Carot14	0.31	-0.14	-0.31	0.36	0.19	0.31	0.03	1.00						
Carot16	0.50**	0.36	0.08	0.28	0.19	-0.05	1.00							
SPAD14	-0.01	0.01	-0.38	0.34	0.47*	1.00								
SPAD16	0.26	0.01	-0.06	0.36	1.00									
BM	0.82***	0.60*	0.00	1.00										
HI	0.26	0.31	1.00											
GY	0.60**	1.00												
GNC	1.00													

*=P<0.05; **=P<0.01; ***=P<0.0001

References

- ANDRADE, F.H. Analysis of growth and yield of maize, sunflower and soybean grown at Balcarce, Argentina. **Field Crops Research**, Amsterdam, v. 41, p. 1-12, 1995.
- ARGENTA, G.; SILVA, P.R.F.; BORTOLINI, C.G.; FORSTHOFER, E.L.; STRIEDER, M.L.; STEFANI, G.F. Relação entre teor de clorofila extraível e leitura do clorofilômetro na folha de milho. In: CONGRESSO NACIONAL DE MILHO E SORGO, 23., 2000, Uberlândia. **Resumos...** Uberlândia: ABMS, 2000. p. 197.
- ARGENTA, G.; SILVA, P.R.F.; SANGOI, L. Arranjo de plantas em milho: análise do estado da arte. **Ciência Rural**, Santa Maria, v. 31, n. 5, p. 1075-1084, 2001.
- ARNON, I. **Mineral nutrition of maize**. Bern: International Potash Institute, 1975. 452 p.
- BARBIE, A.; PROSSER, S.J. Automated analysis of light-element stable isotope ratio mass spectrometry. In: BOUTTON, T.W.; YAMASAKI, S. (Ed.). **Mass spectrometry of soils**. New York: Marcel Decker, 1996. p. 1-46.
- BASSO, C.J.; CERETTA, C.A. Manejo do nitrogênio no milho em sucessão a plantas de cobertura de solo, sob plantio direto. **Revista Brasileira de Ciência do Solo**, Campinas, v. 24, n. 4, p. 905-915, 2000.
- CAMPOSTRINI, E. **Fluorescência da clorofila a: considerações teóricas e aplicações práticas**. Campos dos Goytacazes: UENF, 1988. 34 p.
- CANTARELLA, H. Calagem e adubação do milho. In: BÜLL, L.T.; CANTARELLA, H. (Ed.). **Cultura do milho: fatores que afetam a produtividade**. Piracicaba: POTAFOS, 1993. p. 148-196.
- CANTARELLA, H.; RAIJ, B. van; CAMARGO, C.E.O. Cereais. In: RAIJ, B. van; CANTARELLA, H.; QUAGGIO, J.A.; FURLANI, A.M.C. **Recomendações de adubação e calagem para o Estado de São Paulo**. 2. ed. Campinas: IAC. 1997. p. 45-71 (Boletim Técnico, 100).
- CERETTA, C.A.; BASSO, C.J.; FLECHA, A.M.T.; PAVINATO, P.S.; VIEIRA, F.C.B.; MAI, M.E.M. Manejo da adubação nitrogenada na sucessão aveia preta/milho, no sistema plantio direto. **Revista Brasileira de Ciência do Solo**, Vicosa, v. 26, n. 1, p. 163-171, 2002.
- CIAMPITTI, I.A.; ZHANG, H.; FRIEDEMANN, P.; VYN, T.J. Potential physiological frameworks for mid-season field phenotyping of final plant nitrogen uptake, nitrogen use efficiency, and grain yield in maize. **Crop Science**, Madison, v. 52, p. 2728-2742, 2012.
- COELHO, A.M. **Balço de nitrogênio (15 N) na cultura do milho (*Zea mays* L.) em um Latossolo Vermelho Escuro fase cerrado**. 1987. 142 p. Dissertação (Mestrado em Solos e Nutrição de Plantas) – Escola Superior de Agricultura de Lavras, Lavras, 1987.
- DWYER, L.M.; TOLLENAAR, M.; HOUWING, L. A nondestructive method to monitor leaf greenness in corn. **Canadian Journal of Plant Science**, Ottawa, v. 71, p. 505-509, 1991.

- DWYER, L.M.; ANDERSON, A.M.; MA, B.L.; STEWART, D.W.; TOLLENAAR, M.; GREGORICH, E. Quantifying the nonlinearity in chlorophyll meter response to corn leaf nitrogen concentration. **Canadian Journal of Plant Science**, Ottawa, v. 75, p. 179-182, 1995.
- FANCELLI, A.L. **Nutrição e adubação do milho**. Piracicaba: ESALQ, 2000. 43 p.
- FANCELLI, A.L.; DOURADO NETO, D. **Produção de milho**. 2. ed. Guaíba: Agropecuária, 2004. 360 p.
- FRANÇA, G.E.; COELHO, A.M.; RESENDE, M.; BAHIA FILHO, A.F.C. Parcelamento da adubação nitrogenada em cobertura na cultura do milho irrigado. In: EMBRAPA. Centro Nacional de Pesquisa de Milho e Sorgo. **Relatório técnico anual do Centro Nacional de Pesquisa de Milho e Sorgo**: 1992-1993. Sete Lagoas, 1994. p. 28-29.
- GAVA, G.J.C.; TRIVELIN, P.C.O.; OLIVEIRA, M.W.; HEINRICH, R.; SILVA, M.A. Balanço do nitrogênio da uréia (^{15}N) no sistema solo-planta na implantação da semeadura direta na cultura do milho. **Bragantia**, Campinas, v. 65, p. 477-486, 2006.
- HANWAY, J.J. Growth stages of corn (*Zea mays* L.). **Agronomy Journal**, Madison, v. 55, p. 487-492, 1963.
- JOKELA, W.E.; RANDALL, G.W. Corn yield and residual soil nitrate as affected by time and rate of nitrogen application. **Agronomy Journal**, Madison, v. 81, p. 720-726, 1989.
- MARENCO, R.A.; LOPES, N.F. **Fisiologia vegetal**: fotossíntese, respiração, relações hídricas e nutrição mineral. 2. ed. Viçosa: UFV, 2005. 439 p.
- MARKWELL, J.; OSTERMAN, J.C.; MITCHELL, J.L. Calibration of the Minolta SPAD-502 leaf chlorophyll meter. **Photosynthesis Research**, Dordrecht, v. 46, p. 467-472, 1995.
- MINOLTA CAMERA. **Manual for chlorophyll meter SPAD 502**. Osaka: Radiometric Instruments Divisions, 1989. 22 p.
- MORAN, R.; PORATH, D. Chlorophyll determination in intact tissues using n,n dimethylformamide. **Plant Physiology**, Rockville, v. 65, p. 478-479, 1980.
- NEPTUNE, A.M.L.; CAMPANELLI, A. Efeitos de épocas e modo de aplicação do sulfato de amônio - 15 N, fósforo - 32 P, na quantidade e teores de N, P e K na planta e na folha do milho, na produção, na quantidade de proteína e eficiência do nitrogênio do fertilizante convertido em proteína. **Anais da Escola Superior de Agricultura "Luiz de Queiroz"**, Piracicaba, v. 37, n. 2, p. 1105-1143, 1980.
- PAULETTI, V.; COSTA, L.C. Época de aplicação de nitrogênio no milho cultivado em sucessão à aveia preta no sistema plantio direto. **Ciência Rural**, Santa Maria, v. 30, n. 4, p. 599-603, 2000.
- PESTANA, M.; DAVID, M.; VARENNES, A.; ABADIA, J.; FARIA, E.A. Responses of "Newhall" oranges trees to iron deficiency in hidroponics: effects on leaf chlorophyll, photosynthetic efficiency, and root ferric chelate reductase activity. **Journal of Plant Nutrition**, New York, v. 24, p. 1609-1620, 2001.

PIEKIELEK, W.P.; FOX, R.H. Use of a chlorophyll meter to predict side dress nitrogen requirements for maize. **Agronomy Journal, Madison**, v. 84, n. 1, p. 59-65, 1992.

PIEKIELEK, W.P.; FOX, R.H.; TOTH, J.D.; MACNEAL, K.E. Use of a chlorophyll meter at the early dent stage of corn to evaluate N sufficiency. **Agronomy Journal, Madison**, v. 87, n. 3, p. 403-408, 1995.

PIONNER. **Híbridos de milho: 30F35HR**. Santa Cruz do Sul, 2014. Disponível em: <<http://www.pioneersementes.com.br/DownloadCenter/Catalogo-De-Produtos-Milho-Safrinha-2014.pdf>>. Acesso em: 20 jan. 2015.

RITCHIE, S.W.; HANWAY, J.J.; BENSON, G.O. Como a planta de milho se desenvolve. **Informações Agrônomicas**, Piracicaba, n. 103, p. 1-11, 2003.

SÁ, J.C.M. de. **Manejo de nitrogênio na cultura do milho no sistema plantio direto**. Passo Fundo: Aldeia Norte, 1996. 23 p.

SAS INSTITUTE. **SAS/STAT 9.1: user's guide**. Cary, 2004.

SETTIMI, J.R.; MARANVILLE, J.W. Carbon dioxide assimilation efficiency of maize leaves under nitrogen stress at different stages of plant development. **Soil Science and Plant Analysis**, Philadelphia, v. 29, p. 777-792, 1998.

SOUSA, D.M.G. de; LOBATO, E. Calagem e adubação para culturas anuais e semiperenes. In: _____. (Ed.). **Cerrado: correção do solo e adubação**. Planaltina: Embrapa Cerrados, 2004. p. 283-315.

TRIVELIN, P.C.O.; OLIVEIRA, M.W.; VITTI, A.C.; GAVA, G.J.C.; BENDASSOLLI, J.A. Perdas do nitrogênio da uréia no sistema solo-planta em dois ciclos de cana-de-açúcar. **Pesquisa Agropecuária Brasileira**, Brasília, v. 37, p. 193-201, 2002.

5 GENERAL DISCUSSION

Problems caused to agriculture by drought weather conditions are and will be always present in the world, with more or less intensity. In addition to drought, the other environmental biotic and, also, abiotic stresses together are significant factors that decrease agricultural production. Therefore, studies on crop tolerance related to these stresses and on higher efficiency in the use of environmental resources are extremely important to maintain and increase food production for the growing world population.

5.1 Implications for Agriculture and Science

We can affirm that the drought is a constant problem in the agriculture with large influence in the maize grain production. In this way, the silking antheses interval (ASI) or the period of development of a genotype is very important to decide the best planting date aiming to avoid drought weather conditions in the most sensitive plant development stages, such as the flowering period.

Some maize genotypes present shorter or longer ASI than the other, so it is important to know these periods for each genotype and to perform planting at the best time to avoid the drought conditions.

In Chapter 2 we evaluated the possible traits that govern the nutrient uptake and concentrations between drought and non-drought tolerant hybrids and their influence in grain productivity under different management treatments. In this study we could observe that shorter periods between anthesis and silking and accumulation of more macro-nutrients in the stover, during the dry season, could be some of the mechanisms used by drought tolerant hybrids in achieving greater plant biomass production and possible grain yield. However, all hybrids responded similarly in grain yield to plant density and N rate treatments in the dry or more normal weather year.

In Chapter 3 we investigated the physiological and yield responses of comparable-maturity drought and non-drought tolerant hybrids to varied plant density and N rates. We also evaluated the leaf photosynthesis and transpiration rates of hybrids across the growing season in response to varied plant density and N rate treatments. We observed that there was no evidence that the drought tolerant hybrids were any different than the non-drought tolerant in their response to N fertilizer or to plant density. However, we observed that lower LAI and

higher photosynthesis and transpiration present in the drought tolerant hybrids could be a drought tolerant mechanism used by these hybrids to improve their water use efficiency.

For the two Chapters (2 and 3) we hypothesized that the drought tolerant hybrids could be any different in the nitrogen uptake and grain productivity related to their counterparts with similar maturity. However, the drought tolerant did not show any merit in comparison to the non-drought tolerant related to grain yield in the dry or more normal weather condition seasons. In view of that, future drought tolerant hybrids, developed with biotechnology or transgenic hybrids, could present more advantages in comparison to their counterparts with less drought tolerance.

In Chapter 4 we evaluated methods for crop analysis, SPAD and chlorophyll and carotenoid contents in the maize leaves aiming to verify maize crop conditions in response to environmental factors, such nitrogen fertilization. We also studied the response of maize to nitrogen fertilization in different stages for side-dress fertilizer applications. We concluded that the SPAD device can be used as an efficient method to evaluate the maize nitrogen status in the early growth stages with a strong correlation with chlorophylls, carotenoids and grain yield, mainly after the V16 sample stage. In this study, there was no difference for grain yield in response to the treatments with nitrogen application in different growth stages in side-dress.

APPENDICES

Table 5.1 - Plant heights, and stalk diameters for all hybrids (Hybrid 1 = AQUAmax™ P1151, Hybrid 2 = P1162, Hybrid 3 = AQUAmax™ P1498, and Hybrid 4 = 33D49), both densities (PD1 = 79,000, PD2 = 104,000 pl ha⁻¹), and all N rates (Nr1 = 0 kg N ha⁻¹, Nr2 = 134 kg N ha⁻¹, Nr3 = 202 kg N ha⁻¹, Nr4 = 269 kg N ha⁻¹) in 2012

	Height (cm)				Stalk Diameter (mm)				
	V5	V10	V15	R1	V10	V15	R1	R3	R4
Hybrid									
Hyb 1	47	135	143	174	26.4 a	24.1 a	23.5 ab	22.6 a	22.2 a
Hyb 2	48	136	129	151	25.3 ab	22.6 b	21.4 b	21.0 b	20.4 b
Hyb 3	48	132	128	164	23.7 b	22.5 b	21.9 b	21.3 b	20.9 b
Hyb 4	45	131	127	178	24.3 b	23.5 ab	22.6 ab	21.6 ab	21.3 ab
PD									
PD 1	47	135	136 a	171 a	26.0 a	24.2 a	23.4 a	22.5 a	22.1 a
PD 2	47	132	128 b	162 b	23.9 b	22.1 b	21.4 b	20.8 b	20.3 b
Nr									
Nr 1	46	133	128	166	24.7	22.7 b	21.9 b	21.4	21
Nr 2	47	133	134	169	24.8	23.0 ab	22.3 ab	21.6	21
Nr 3	47	136	132	166	25.2	23.6 a	22.7 a	22	21.4
Nr 4	47	133	133	167	25	23.4 ab	22.6 ab	21.6	21.5
Anova									
Hyb	ns	ns	ns	ns	*	*	**	**	*
PD	ns	ns	**	**	***	***	***	***	***
Nr	ns	ns	ns	ns	ns	*	*	ns	ns
Hyb x PD	ns	*	*	*	ns	ns	*	ns	*
Hyb x Nr	ns	ns	ns	ns	ns	ns	ns	ns	ns
PD x Nr	ns	ns	ns	ns	ns	ns	ns	ns	ns
Hyb x PD x Nr	ns	ns	ns	ns	ns	ns	ns	ns	ns

ns = not significant; *=P<0.05; **=P<0.01; ***=P<0.0001

Table 5.2 - Plant heights, and stalk diameters for all hybrids (Hybrid 1 = AQUAmax™ P1151, Hybrid 2 = P1162, Hybrid 3 = AQUAmax™ P1498, and Hybrid 4 = 33D49), both densities (PD1 = 78,000, PD2 = 99,000 pl ha⁻¹), and all N rates (Nr1 = 0 kg N ha⁻¹, Nr2 = 134 kg N ha⁻¹, Nr3 = 202 kg N ha⁻¹, Nr4 = 269 kg N ha⁻¹) in 2013

	Height (cm)			Stalk Diameter (mm)		
	V5	V10	V15	V10	V12	V14
Hybrid						
Hyb 1	64 b	133 a	222 b	24.7 a	24.0 a	25.6 a
Hyb 2	70 a	137 a	216 b	24.4 a	23.4 ab	25.7 a
Hyb 3	63 b	128 b	229 a	23.6 b	23.2 b	23.5 b
Hyb 4	62 b	126 b	227 a	24.3 a	23.8 ab	25.3 a
PD						
PD 1	64	130	226	25.1 a	24.7 a	26.0 a
PD 2	65	132	222	23.4 b	22.5 b	24.2 b
Nr						
Nr 1	63	119 b	196 b	22.7 b	21.1 c	23.2 c
Nr 2	65	133 a	230 a	24.5 a	24.0 b	25.2 b
Nr 3	65	135 a	234 a	25.0 a	25.0 a	25.1 b
Nr 4	65	136 a	235 a	25.0 a	25.0 a	26.7 a
Anova						
Hyb	**	**	*	**	*	**
PD	ns	ns	ns	**	**	**
Nr	ns	**	**	**	**	**
Hyb x PD	ns	ns	ns	*	ns	ns
Hyb xNr	ns	ns	ns	ns	ns	ns
PD x Nr	ns	ns	ns	ns	ns	ns
Hyb x PD x Nr	ns	ns	ns	ns	ns	ns

ns = not significant; *=P<0.05; **=P<0.01; ***=P<0.0001

Table 5.3 - Mean separation test for plant height (cm) at V10, V15, and R1 growth stages, and stalk diameter (mm) at R1 and R4 growth stages, for the factorial Hybrid x PD for all four hybrids (Hybrid 1 = AQUAmax™ P1151, Hybrid 2 = P1162, Hybrid 3 =AQUAmax™ P1498, Hybrid 4 = 33D49) and each plant density level (PD1 = 79,000; and PD2 = 104,000 pl ha⁻¹) in 2012

Hybrid	Height V10 (cm)		Height V15 (cm)		Height R1 (cm)		Stalk R1 (mm)		Stalk R4 (mm)	
	PD1	PD2	PD1	PD2	PD1	PD2	PD1	PD2	PD1	PD2
Hyb 1	127.2 Ba	135.1 Aa	135.5 Aa	140.4 Aa	164.4 Ba	170.9 Aa	24.1 Aa	23.0 Ab	22.6 Aa	21.7 Ab
Hyb 2	145.4 Aa	131.7 Ab	141.6 Aa	125.7 Bb	165.3 Ba	145.8 Bb	22.7 Ba	20.0 Cb	21.5 Ba	19.2 Cb
Hyb 3	134.5 ABa	131.1 Aa	131.7 Aa	124.7 Ba	168.4 Ba	161.4 Aa	22.7 Ba	21.2 Bb	21.7 Ba	20.1 Bb
Hyb 4	133.2 Ba	131.0 Aa	135.2 Aa	119.8 Bb	187.0 Aa	170.8 Ab	24.0 Aa	21.3 Bb	22.6 Aa	20.3 Bb

Different lower case letter in the rows: the same hybrid differs in Plant densities. Different uppercase letter in the columns: the hybrids differ in the same PD

Table 5.4 - Mean separation test for ZHI (Zink harvest index), for the factorial Hybrid x N rate, and Stalk V10 (Stalk diameter at V10 growth stage), for the factorial Hybrid x Plant density (PD), for all four hybrids (Hybrid 1 = AQUAmax™ P1151, Hybrid 2 = P1162, Hybrid 3 =AQUAmax™ P1498, Hybrid 4 = 33D49), each plant density level (PD1 = 78,000; and PD2 = 99,000 pl ha⁻¹), and four N rates (Nr1 = 0, Nr2 = 134, Nr3 = 202, and Nr4 = 269 kg ha⁻¹), respectively, in 2013

Hybrid	ZHI				Stalk V10 (mm)	
	Nr1	Nr2	Nr3	Nr4	PD1	PD2
Hyb 1	0.451 Ab	0.605 Aa	0.608 BCa	0.647 Aa	26.07 Aa	23.45 Ab
Hyb 2	0.523 Ab	0.605 Aab	0.534 Cb	0.620 Aa	25.49 Aa	23.36 Ab
Hyb 3	0.455 Ab	0.634 Aa	0.691 Aa	0.689 Aa	24.19 Ba	23.04 Ab
Hyb 4	0.371 Bb	0.560 Ab	0.644 ABa	0.616 Aab	24.83 Ba	23.41 Ab

Different lower case letter in the rows: the same Hybrid differs in the all N rates or Plant densities. Different uppercase letter in the columns: hybrid treatments differ in the same N rate or PD

Table 5.5 - Mean SPAD for all hybrids (Hybrid 1 = AQUAmax™ P1151, Hybrid 2 = P1162, Hybrid 3 = AQUAmax™ P1498, and Hybrid 4 = 33D49) at both densities (PD1 = 79,000 pl ha⁻¹, PD2 = 104,000 pl ha⁻¹) and at all N rates (Nr1 = 0 kg N ha⁻¹, Nr2 = 134 kg N ha⁻¹, Nr3 = 202 kg N ha⁻¹, Nr4 = 269 kg N ha⁻¹) in 2012

	SPAD						
	V10	V12	V15	R1	R3	R4	R5
Hybrid							
Hyb 1	55.3	53.9	50.8	48.4	49.7 b	47.9 ab	39.4
Hyb 2	51.9	49	48.2	45.9	48.4 b	45.2 b	37.6
Hyb 3	54.3	53.5	49.6	49.8	50.0 ab	47.2 ab	42.1
Hyb 4	56.4	53.4	50.1	49.4	53.5 a	51.3 a	45.5
PD							
PD 1	55.0 a	53.6 a	50.7 a	49.3 a	51.8 a	49.1 a	41.7
PD 2	53.9 b	51.4 b	48.7 b	47.4 b	49.0 b	46.0 b	40.6
Nr							
Nr 1	53.8	51.9 b	48.1 b	46.6 b	48.0 b	43.9 b	35.8 b
Nr 2	54.6	-	48.8 ab	48.8 a	50.8 a	48.7 a	-
Nr 3	54.8	53.0 a	50.1 a	48.9 a	50.9 a	49.0 a	46.5 a
Nr 4	54.7	-	50.7 a	49.2 a	51.9 a	50.1 a	-
Anova							
Hyb	ns	ns	ns	ns	*	*	ns
PD	**	***	**	**	***	***	ns
Nr	ns	*	**	**	***	***	***
Hyb x PD	ns	ns	ns	ns	ns	ns	ns
Hyb x Nr	*	ns	ns	ns	ns	ns	ns
PD x Nr	ns	ns	ns	ns	ns	ns	ns
Hyb x PD x Nr	ns	ns	ns	ns	ns	ns	ns

ns = not significant; *=P<0.05; **=P<0.01; ***=P<0.0001

Table 5.6 - Mean SPAD for all hybrids (Hybrid 1 = AQUAmax™ P1151, Hybrid 2 = P1162, Hybrid 3 = AQUAmax™ P1498, and Hybrid 4 = 33D49) at both densities (PD1 = 78,000, PD2 = 99,000 pl ha⁻¹) at all N rates (Nr1 = 0 kg N ha⁻¹, Nr2 = 134 kg N ha⁻¹, Nr3 = 202 kg N ha⁻¹, Nr4 = 269 kg N ha⁻¹) in 2013

	SPAD		
	V10	V12	V14
Hybrid			
Hyb 1	55.0 b	46.3 a	51.7 a
Hyb 2	50.5 d	43.6 b	49.2 b
Hyb 3	53.4 c	47.5 a	51.8 a
Hyb 4	56.3 a	44.6 b	50.1 a
PD			
PD 1	54.8 a	46.6 a	52.5 a
PD 2	52.8 b	44.4 b	49.0 b
Nr			
Nr 1	46.5 b	37.9 b	39.2 c
Nr 2	55.8 a	47.3 a	54.4 ab
Nr 3	56.1 a	48.4 a	53.7 b
Nr 4	56.8 a	48.4 a	55.6 a
Anova			
Hyb	**	**	**
PD	**	**	**
Nr	**	**	**
Hyb x PD	ns	ns	ns
Hyb xNr	ns	ns	ns
PD x Nr	ns	ns	ns
Hyb x PD x Nr	ns	ns	ns

ns = not significant; *=P<0.05; **=P<0.01; ***=P<0.0001

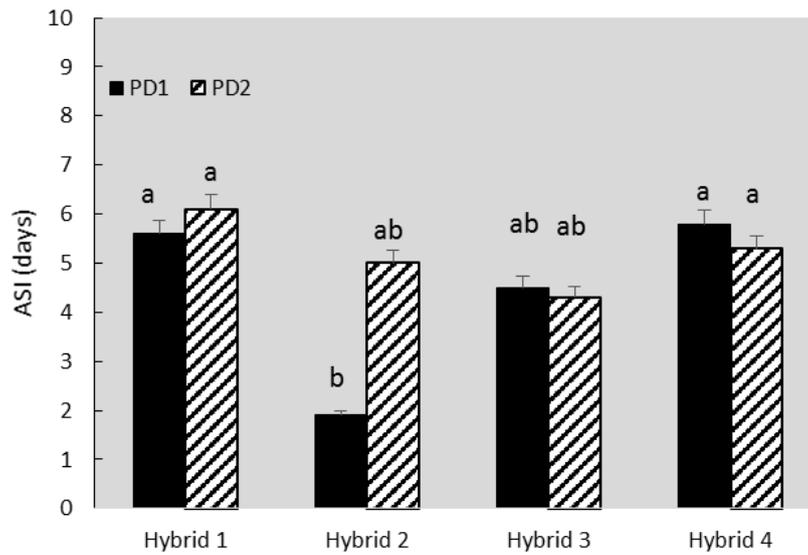


Figure 5.1 - Anthesis-silking interval (ASI) (days) for the factorial Hybrid x PD at physiological maturity for all maize hybrids (Hybrid 1 = AQUAmax™ P1151, Hybrid 2 = P1162, Hybrid 3 = AQUAmax™ P1498, Hybrid 4 = 33D49, and Hybrid 5 = P1184) grown at two plant densities (PD1=79,000, PD2=104,000 pl ha⁻¹) and four N rates (Nr1 = 0, Nr2 = 134, Nr3 = 202, and Nr4 = 269 kg ha⁻¹) in 2012

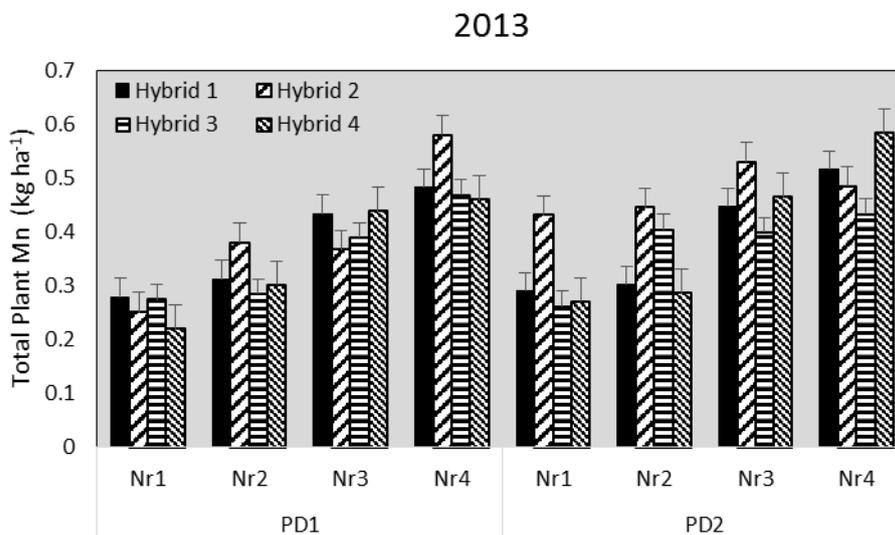


Figure 5.2 - Total plant Mn (kg ha⁻¹) for the factorial Hybrid x PD x N rate at physiological maturity for all four hybrids (Hybrid 1 = AQUAmax™ P1151, Hybrid 2 = P1162, Hybrid 3 = AQUAmax™ P1498, Hybrid 4 = 33D49), each plant density level (PD1 = 78,000; and PD2 = 99,000 pl ha⁻¹), and all four N rate levels (Nr1 = 0, Nr2 = 134, Nr3 = 202, and Nr4 = 269 kg N sidedress ha⁻¹), in 2013

Table 5.7 - Zinc partitioning (kg ha^{-1} dry weight) into grain, cob, and stover components, and zinc harvest index (ZnHI) at physiological maturity for all maize hybrids (Hybrid 1 = AQUAmax™ P1151, Hybrid 2 = P1162, Hybrid 3 = AQUAmax™ P1498, Hybrid 4 = 33D49) grown at two plant densities (PD1 = 79,000, PD2 = 104,000 pl ha^{-1}) and four N rates (Nr1 = 0, Nr2 = 134, Nr3 = 202, and Nr4 = 269 kg ha^{-1}) in 2012

	Grain Zn (kg ha^{-1})	Cob Zn (kg ha^{-1})	Stover Zn (kg ha^{-1})	Total Zn (kg ha^{-1})	ZnHI
Hybrid					
Hyb 1	0.09 c	0.02	0.22 a	0.34 c	0.26 c
Hyb 2	0.11 c	0.04	0.19 b	0.35 c	0.33 b
Hyb 3	0.16 b	0.05	0.24 a	0.45 a	0.34 b
Hyb 4	0.19 a	0.03	0.18 b	0.40 b	0.48 a
PD					
PD 1	0.13	0.03	0.21	0.38	0.34
PD 2	0.15	0.04	0.21	0.4	0.36
Nr					
Nr 1	0.12	0.03	0.17 c	0.32 c	0.38
Nr 2	0.13	0.04	0.21 b	0.40 b	0.34
Nr 3	0.14	0.03	0.22 ab	0.40 b	0.34
Nr 4	0.16	0.04	0.24 a	0.44 a	0.34
Anova					
Hyb	**	na	**	**	**
PD	ns	na	ns	ns	ns
Nr	ns	na	**	**	ns
Hyb x PD	**	na	*	*	**
Hyb xNr	ns	na	ns	*	ns
PD x Nr	ns	na	ns	ns	ns
Hyb x PD x Nr	**	na	ns	ns	**

ns = not significant; na = not available; *= $P < 0.05$; **= $P < 0.01$; ***= $P < 0.0001$.

Cob nutrient concentration for the same treatment in reps 2 and 3 was estimated from rep 1 data but adjusted for each individual plot cob weight so statistical analysis was not possible

Table 5.8 - Zinc partitioning (kg ha⁻¹ dry weight) into grain, cob, and stover components, and zinc harvest index (ZnHI) at physiological maturity for all maize hybrids (Hybrid 1 = AQUAmax™ P1151, Hybrid 2 = P1162, Hybrid 3 = AQUAmax™ P1498, Hybrid 4 = 33D49) grown at two plant densities (PD1 = 79,000, PD2 = 104,000 pl ha⁻¹) and four N rates (Nr1 = 0, Nr2 = 134, Nr3 = 202, and Nr4 = 269 kg ha⁻¹) in 2013

	Grain Zn (kg ha ⁻¹)	Cob Zn (kg ha ⁻¹)	Stover Zn (kg ha ⁻¹)	Total Zn (kg ha ⁻¹)	ZnHI
Hybrid					
Hyb 1	0.18 a	0.01	0.11	0.32 a	0.58 b
Hyb 2	0.19 a	0.03	0.12	0.34 a	0.57 b
Hyb 3	0.19 a	0.01	0.1	0.31 a	0.62 a
Hyb 4	0.15 b	0.01	0.1	0.27 b	0.55 b
PD					
PD 1	0.18	0.02	0.11	0.31	0.58
PD 2	0.18	0.02	0.11	0.31	0.57
Nr					
Nr 1	0.12 c	0.02	0.13 a	0.27 c	0.45 c
Nr 2	0.17 b	0.02	0.10 b	0.29 bc	0.60 b
Nr 3	0.19 b	0.02	0.11 b	0.32 ab	0.62 ab
Nr 4	0.23 a	0.02	0.11 b	0.36 a	0.64 a
Anova					
Hyb	*	na	ns	**	**
PD	ns	na	ns	ns	ns
Nr	**	na	**	**	**
Hyb x PD	ns	na	ns	ns	ns
Hyb xNr	ns	na	ns	ns	**
PD x Nr	ns	na	ns	ns	ns
Hyb x PD x Nr	ns	na	ns	ns	ns

ns = not significant; na = not available; *=P<0.05; **=P<0.01; ***=P<0.0001.

Cob nutrient concentration for the same treatment in reps 2 and 3 was estimated from rep 1 data but adjusted for each individual plot cob weight so statistical analysis was not possible

Table 5.9 - Iron partitioning (kg ha^{-1} dry weight) into grain, cob, and stover components, and iron harvest index (FeHI) at physiological maturity for all maize hybrids (Hybrid 1 = AQUAmax™ P1151, Hybrid 2 = P1162, Hybrid 3 = AQUAmax™ P1498, Hybrid 4 = 33D49) grown at two plant densities (PD1 = 79,000, PD2 = 104,000 pl ha^{-1}) and four N rates (Nr1 = 0, Nr2 = 134, Nr3 = 202, and Nr4 = 269 kg ha^{-1}) in 2012

	Grain Fe (kg ha^{-1})	Cob Fe (kg ha^{-1})	Stover Fe (kg ha^{-1})	Total Fe (kg ha^{-1})	FeHI
Hybrid					
Hyb 1	0.07 c	0.01	1.25	1.32	0.06 c
Hyb 2	0.08 c	0.01	1.25	1.35	0.05 c
Hyb 3	0.13 b	0.01	1.3	1.43	0.10 b
Hyb 4	0.16 a	0.01	1.17	1.34	0.13 a
PD					
PD 1	0.11	0.01	1.2	1.31	0.08
PD 2	0.11	0.01	1.3	1.41	0.09
Nr					
Nr 1	0.1	0.01	1.27	1.38	0.08
Nr 2	0.11	0.01	1.28	1.4	0.09
Nr 3	0.11	0.01	1.27	1.4	0.08
Nr 4	0.11	0.01	1.15	1.3	0.1
Anova					
Hyb	**	na	ns	ns	**
PD	ns	na	ns	ns	ns
Nr	ns	na	ns	ns	ns
Hyb x PD	ns	na	ns	ns	ns
Hyb x Nr	ns	na	ns	ns	ns
PD x Nr	**	na	ns	ns	ns
Hyb x PD x Nr	ns	na	ns	ns	ns

ns = not significant; na = not available; *= $P < 0.05$; **= $P < 0.01$; ***= $P < 0.0001$.

Cob nutrient concentration for the same treatment in reps 2 and 3 was estimated from rep 1 data but adjusted for each individual plot cob weight so statistical analysis was not possible

Table 5.10 - Iron partitioning (kg ha⁻¹ dry weight) into grain, cob, and stover components, and iron harvest index (FeHI) at physiological maturity for all maize hybrids (Hybrid 1 = AQUAmax™ P1151, Hybrid 2 = P1162, Hybrid 3 = AQUAmax™ P1498, Hybrid 4 = 33D49) grown at two plant densities (PD1 = 79,000, PD2 = 104,000 pl ha⁻¹) and four N rates (Nr1 = 0, Nr2 = 134, Nr3 = 202, and Nr4 = 269 kg ha⁻¹) in 2013

	Grain Fe (kg ha ⁻¹)	Cob Fe (kg ha ⁻¹)	Stover Fe (kg ha ⁻¹)	Total Fe (kg ha ⁻¹)	FeHI
Hybrid					
Hyb 1	0.15	0.01	0.77 b	0.93 b	0.16 bc
Hyb 2	0.14	0.01	0.89 a	1.05 a	0.13 c
Hyb 3	0.16	0.01	0.55 c	0.72 c	0.23 a
Hyb 4	0.13	0.01	0.56 c	0.69 c	0.19 b
PD					
PD 1	0.14	0.01	0.65 b	0.80 b	0.18
PD 2	0.15	0.01	0.74 a	0.90 a	0.17
Nr					
Nr 1	0.07 c	0.01	0.67	0.76 b	0.09 c
Nr 2	0.15 b	0.01	0.73	0.89 a	0.18 b
Nr 3	0.17 ab	0.01	0.72	0.90 a	0.20 b
Nr 4	0.19 a	0.01	0.65	0.85 ab	0.24 a
Anova					
Hyb	ns	na	**	**	**
PD	ns	na	*	**	ns
Nr	**	na	ns	*	**
Hyb x PD	ns	na	ns	ns	ns
Hyb xNr	ns	na	ns	ns	ns
PD x Nr	ns	na	ns	ns	ns
Hyb x PD x Nr	ns	na	ns	ns	ns

ns = not significant; na = not available; *=P<0.05; **=P<0.01; ***=P<0.0001.

Cob nutrient concentration for the same treatment in reps 2 and 3 was estimated from rep 1 data but adjusted for each individual plot cob weight so statistical analysis was not possible

Table 5.11 - Manganese partitioning (kg ha⁻¹ dry weight) into grain, cob, and stover components, and manganese harvest index (MnHI) at physiological maturity for all maize hybrids (Hybrid 1 = AQUAmax™ P1151, Hybrid 2 = P1162, Hybrid 3 = AQUAmax™ P1498, Hybrid 4 = 33D49) grown at two plant densities (PD1 = 79,000, PD2 = 104,000 pl ha⁻¹) and four N rates (Nr1 = 0, Nr2 = 134, Nr3 = 202, and Nr4 = 269 kg ha⁻¹) in 2012

	Grain Mn (kg ha ⁻¹)	Cob Mn (kg ha ⁻¹)	Stover Mn (kg ha ⁻¹)	Total Mn (kg ha ⁻¹)	MnHI
Hybrid					
Hyb 1	0.025 c	0.008	0.582	0.618	0.05 b
Hyb 2	0.029 c	0.013	0.599	0.638	0.04 b
Hyb 3	0.048 b	0.018	0.653	0.720	0.07 a
Hyb 4	0.056 a	0.012	0.647	0.715	0.08 a
PD					
PD 1	0.038	0.011	0.615	0.665	0.06
PD 2	0.041	0.014	0.626	0.681	0.06
Nr					
Nr 1	0.034	0.012	0.464 c	0.509 c	0.07
Nr 2	0.041	0.012	0.608 b	0.662 b	0.07
Nr 3	0.039	0.012	0.686 ab	0.738 ab	0.05
Nr 4	0.044	0.016	0.724 a	0.784 a	0.06
Anova					
Hyb	**	na	ns	ns	**
PD	ns	na	ns	ns	ns
Nr	ns	na	**	**	ns
Hyb x PD	ns	na	ns	ns	ns
Hyb x Nr	ns	na	ns	ns	ns
PD x Nr	ns	na	ns	ns	ns
Hyb x PD x Nr	*	na	ns	ns	ns

ns = not significant; na = not available; *= $P < 0.05$; **= $P < 0.01$; ***= $P < 0.0001$.

Cob nutrient concentration for the same treatment in reps 2 and 3 was estimated from rep 1 data but adjusted for each individual plot cob weight so statistical analysis was not possible

Table 5.12 - Manganese partitioning (kg ha⁻¹ dry weight) into grain, cob, and stover components, and manganese harvest index (MnHI) at physiological maturity for all maize hybrids (Hybrid 1 = AQUAmax™ P1151, Hybrid 2 = P1162, Hybrid 3 = AQUAmax™ P1498, Hybrid 4 = 33D49) grown at two plant densities (PD1 = 79,000, PD2 = 104,000 pl ha⁻¹) and four N rates (Nr1 = 0, Nr2 = 134, Nr3 = 202, and Nr4 = 269 kg ha⁻¹) in 2013

	Grain Mn (kg ha ⁻¹)	Cob Mn (kg ha ⁻¹)	Stover Mn (kg ha ⁻¹)	Total Mn (kg ha ⁻¹)	MnHI
Hybrid					
Hyb 1	0.038	0.004	0.342 b	0.383 b	0.09 a
Hyb 2	0.03	0.005	0.400 a	0.434 a	0.07b
Hyb 3	0.035	0.006	0.324 b	0.365 b	0.09 b
Hyb 4	0.03	0.004	0.345 b	0.378 b	0.07 b
PD					
PD 1	0.033	0.004	0.333 b	0.370 b	0.09
PD 2	0.032	0.005	0.370 a	0.410 a	0.08
Nr					
Nr 1	0.018 c	0.004	0.260 d	0.285 d	0.06 b
Nr 2	0.034 b	0.004	0.302 c	0.340 c	0.10 a
Nr 3	0.037 ab	0.005	0.392 b	0.434 b	0.08 a
Nr 4	0.046 a	0.005	0.453 a	0.501 a	0.08 a
Anova					
Hyb	ns	na	**	**	*
PD	ns	na	*	**	ns
Nr	**	na	**	**	**
Hyb x PD	ns	na	ns	ns	ns
Hyb xNr	ns	na	ns	ns	ns
PD x Nr	ns	na	ns	ns	ns
Hyb x PD x Nr	ns	na	ns	*	ns

ns = not significant; na = not available; *=P<0.05; **=P<0.01; ***=P<0.0001.

Cob nutrient concentration for the same treatment in reps 2 and 3 was estimated from rep 1 data but adjusted for each individual plot cob weight so statistical analysis was not possible

Table 5.13 - Copper partitioning (kg ha⁻¹ dry weight) into grain, cob, and stover components, and copper harvest index (CuHI) at physiological maturity for all maize hybrids (Hybrid 1 = AQUAmaxTM P1151, Hybrid 2 = P1162, Hybrid 3 = AQUAmaxTM P1498, Hybrid 4 = 33D49) grown at two plant densities (PD1 = 79,000, PD2 = 104,000 pl ha⁻¹) and four N rates (Nr1 = 0, Nr2 = 134, Nr3 = 202, and Nr4 = 269 kg ha⁻¹) in 2012

	Grain Cu (kg ha ⁻¹)	Cob Cu (kg ha ⁻¹)	Stover Cu (kg ha ⁻¹)	Total Cu (kg ha ⁻¹)	CuHI
Hybrid					
Hyb 1	0.006 c	0.003	0.039 a	0.048 c	0.13 c
Hyb 2	0.009 b	0.002	0.029 b	0.041 d	0.25 a
Hyb 3	0.008 b	0.003	0.041 a	0.053 bc	0.16 c
Hyb 4	0.012 a	0.004	0.042 a	0.058 a	0.21 b
PD					
PD 1	0.008 b	0.003	0.039	0.05	0.17 b
PD 2	0.010 a	0.003	0.037	0.05	0.20 a
Nr					
Nr 1	0.008	0.002	0.029 b	0.040 b	0.22 a
Nr 2	0.009	0.003	0.040 a	0.053 a	0.18 ab
Nr 3	0.009	0.003	0.041 a	0.053 a	0.16 b
Nr 4	0.009	0.003	0.041 a	0.054 a	0.18 b
Anova					
Hyb	**	na	**	**	**
PD	**	na	ns	ns	*
Nr	ns	na	**	**	*
Hyb x PD	*	na	**	ns	*
Hyb x Nr	ns	na	ns	ns	ns
PD x Nr	ns	na	ns	ns	ns
Hyb x PD x Nr	ns	na	ns	ns	ns

ns = not significant; na = not available; * = P < 0.05; ** = P < 0.01; *** = P < 0.0001.

Cob nutrient concentration for the same treatment in reps 2 and 3 was estimated from rep 1 data but adjusted for each individual plot cob weight so statistical analysis was not possible

Table 5.14 - Copper partitioning (kg ha^{-1} dry weight) into grain, cob, and stover components, and copper harvest index (CuHI) at physiological maturity for all maize hybrids (Hybrid 1 = AQUAmax™ P1151, Hybrid 2 = P1162, Hybrid 3 = AQUAmax™ P1498, Hybrid 4 = 33D49) grown at two plant densities (PD1 = 79,000, PD2 = 104,000 pl ha^{-1}) and four N rates (Nr1 = 0, Nr2 = 134, Nr3 = 202, and Nr4 = 269 kg ha^{-1}) in 2013

	Grain Cu (kg ha^{-1})	Cob Cu (kg ha^{-1})	Stover Cu (kg ha^{-1})	Total Cu (kg ha^{-1})	CuHI
Hybrid					
Hyb 1	0.018 ab	0.003	0.032 a	0.052 ab	0.34 bc
Hyb 2	0.022 a	0.002	0.031 a	0.055 a	0.38 ab
Hyb 3	0.019 ab	0.002	0.025 b	0.046 c	0.40 a
Hyb 4	0.016 b	0.003	0.029 a	0.048 bc	0.33 c
PD					
PD 1	0.017	0.003	0.03	0.049	0.35
PD 2	0.02	0.002	0.03	0.052	0.37
Nr					
Nr 1	0.010 b	0.001	0.015 c	0.026 c	0.37
Nr 2	0.020 a	0.003	0.031 b	0.054 b	0.36
Nr 3	0.021 a	0.003	0.034 ab	0.058 ab	0.36
Nr 4	0.023 a	0.003	0.036 a	0.062 a	0.36
Anova					
Hyb	*	na	**	*	*
PD	ns	na	ns	ns	ns
Nr	**	na	**	**	ns
Hyb x PD	ns	na	ns	ns	ns
Hyb x Nr	ns	na	ns	ns	ns
PD x Nr	ns	na	ns	ns	ns
Hyb x PD x Nr	ns	na	ns	ns	ns

ns = not significant; na = not available; *= $P < 0.05$; **= $P < 0.01$; ***= $P < 0.0001$.

Cob nutrient concentration for the same treatment in reps 2 and 3 was estimated from rep 1 data but adjusted for each individual plot cob weight so statistical analysis was not possible

Table 5.15 - Grain Mn content for the factorial Hybrid x PD x N rate for all four hybrids (Hybrid 1 = AQUAmax™ P1151, Hybrid 2 = P1162, Hybrid 3 =AQUAmax™ P1498, Hybrid 4 = 33D49), each plant density level (PD1 = 79,000; and PD2 = 104,000 pl ha⁻¹), and four N rates (Nr1 = 0, Nr2 = 134, Nr3 = 202, and Nr4 = 269 kg ha⁻¹), respectively, in 2012

Grain Mn (kg ha⁻¹)				
Hybrid	Nr1	Nr2	Nr3	Nr4
Hyb 1				
PD1	0.023	0.014	0.025	0.03
PD2	0.035	0.027	0.02	0.03
Hyb 2				
PD1	0.02	0.03	0.04	0.037
PD2	0.02	0.026	0.023	0.034
Hyb 3				
PD1	0.046	0.053	0.031	0.042
PD2	0.0369	0.05	0.064	0.062
Hyb 4				
PD1	0.063	0.06	0.047	0.055
PD2	0.031	0.07	0.064	0.062

Table 5.16 - Total Mn uptake for the factorial Hybrid x PD x N rate for all four hybrids (Hybrid 1 = AQUAmax™ P1151, Hybrid 2 = P1162, Hybrid 3 =AQUAmax™ P1498, Hybrid 4 = 33D49), each plant density level (PD1 = 78,000; and PD2 = 99,000 pl ha⁻¹), and four N rates (Nr1 = 0, Nr2 = 134, Nr3 = 202, and Nr4 = 269 kg ha⁻¹), respectively, in 2013

Total Mn uptake (kg ha⁻¹)				
Hybrid	Nr1	Nr2	Nr3	Nr4
Hyb 1				
PD1	0.280	0.313	0.434	0.483
PD2	0.290	0.303	0.447	0.516
Hyb 2				
PD1	0.251	0.381	0.367	0.579
PD2	0.4311	0.445	0.53	0.485
Hyb 3				
PD1	0.275	0.285	0.389	0.469
PD2	0.262	0.404	0.399	0.433
Hyb 4				
PD1	0.22	0.302	0.439	0.46
PD2	0.2712	0.287	0.465	0.583

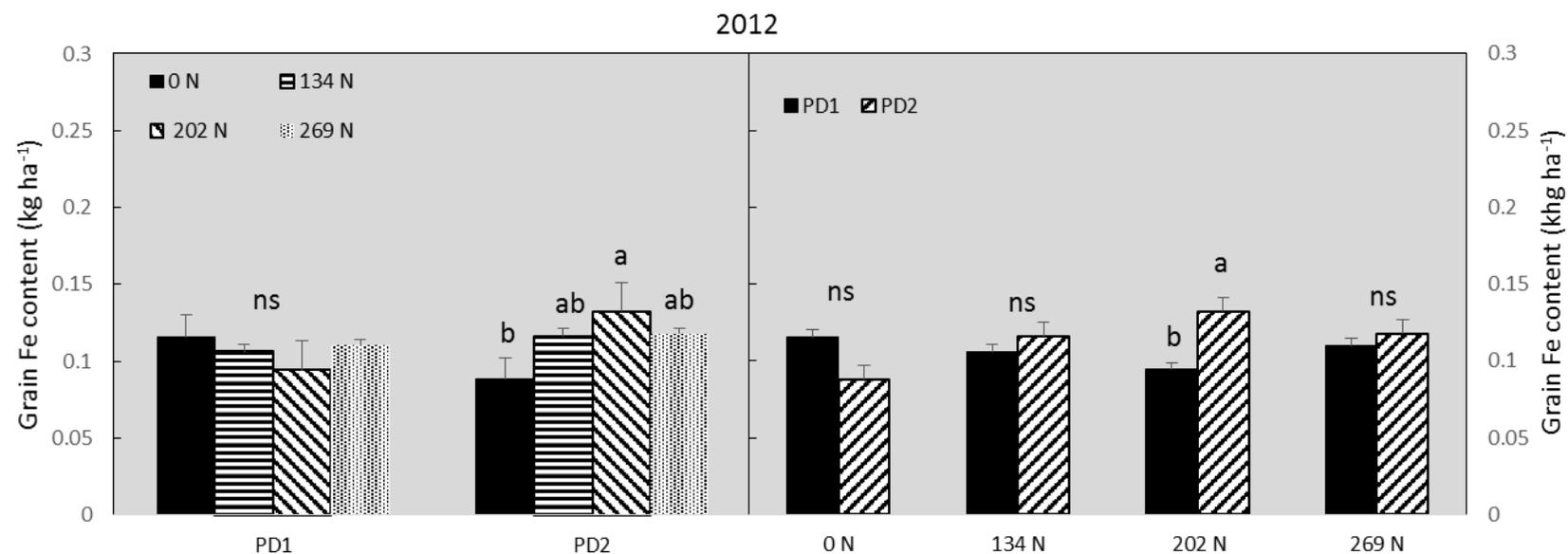


Figure 5.3 - Mean separation test for grain Fe content (kg ha^{-1}) for the factorial PD \times N rate at physiological maturity for each plant density level (PD1 = 79,000; and PD2 = 104,000 pl ha^{-1}), and all four N rate levels (Nr1 = 0, Nr2 = 134, Nr3 = 202, and Nr4 = 269 $\text{kg N sidedress ha}^{-1}$), when averaged across all 4 hybrids in 2012

Table 5.17. Total ZHI (Zinc harvest index), and grain Zn content (kg ha⁻¹) for the factorial Hybrid x PD x N rate for all four hybrids (Hybrid 1 = AQUAmax™ P1151, Hybrid 2 = P1162, Hybrid 3 =AQUAmax™ P1498, Hybrid 4 = 33D49), each plant density level (PD1 = 79,000; and PD2 = 104,000 pl ha⁻¹), and four N rates (Nr1 = 0, Nr2 = 134, Nr3 = 202, and Nr4 = 269 kg ha⁻¹), respectively, in 2012

2012 Hybrid	ZHI				Grain Zn content (kg ha ⁻¹)			
	Nr1	Nr2	Nr3	Nr4	Nr1	Nr2	Nr3	Nr4
Hyb 1								
PD1	0.291	0.219	0.231	0.301	0.088	0.068	0.7	0.103
PD2	0.371	0.236	0.176	0.227	0.109	0.09	0.073	0.09
Hyb 2								
PD1	0.334	0.358	0.373	0.366	0.09	0.095	0.149	0.157
PD2	0.337	0.301	0.319	0.2696	0.094	0.095	0.107	0.114
Hyb 3								
PD1	0.392	0.31	0.198	0.221	0.14	0.138	0.078	0.113
PD2	0.338	0.349	0.485	0.447	0.121	0.157	0.242	0.304
Hyb 4								
PD1	0.549	0.463	0.389	0.424	0.221	0.202	0.174	0.182
PD2	0.411	0.512	0.551	0.499	0.124	0.222	0.244	0.194

Table 5.18 - Mean separation test for stover Zn content (kg ha⁻¹), grain Zn content (kg ha⁻¹), grain Cu content (kg ha⁻¹), stover Cu content (kg ha⁻¹), and CuHI (copper harvest index) for the factorial Hybrid x PD, for all four hybrids (Hybrid 1 = AQUAmaxTM P1151, Hybrid 2 = P1162, Hybrid 3 =AQUAmaxTM P1498, Hybrid 4 = 33D49), and each plant density level (PD1 = 79,000; and PD2 = 104,000 pl ha⁻¹), when averaged across all 4 N rates in 2012

2012 Hybrid	Stover Zn		Grain Zn		Grain Cu		Stover Cu		CuHI	
	PD1	PD2	PD1	PD2	PD1	PD2	PD1	PD2	PD1	PD2
Hyb 1	0.206 b	0.244 a	0.082 a	0.091 a	0.006 a	0.006 a	0.038 B a	0.040 A a	0.121 a	0.133 a
Hyb 2	0.198 a	0.193 a	0.123 a	0.101 a	0.010 a	0.009 a	0.029 C a	0.030 B a	0.264 a	0.240 a
Hyb 3	0.257 a	0.227 a	0.117 b	0.206 a	0.006 b	0.010 a	0.042 Ab a	0.041 A a	0.128 b	0.184 a
Hyb 4	0.199 a	0.163b	0.195 a	0.196 a	0.008 b	0.010 a	0.048 A a	0.037 A b	0.165 b	0.249 a

Different lower case letter in the rows: the same hybrid differs in plant densities. Different uppercase letter in the columns: hybrids differ in the same plant density at $p < 0.05$