

**UNIVERSIDADE ESTADUAL PAULISTA “JÚLIO DE MESQUITA FILHO”
FACULDADE DE CIÊNCIAS AGRÁRIAS E VETERINÁRIAS
CÂMPUS DE JABOTICABAL**

**CARACTERIZAÇÃO DE SINTÉTICOS DE MILHO QUANTO À
EFICIÊNCIA DE USO DO NITROGÊNIO**

**Camila Baptista do Amaral
Engenheira Agrônoma**

2018

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Camila Baptista do Amaral

Orientador: Prof. Dr. Gustavo Vitti Mõro

Tese apresentada à Faculdade de Ciências Agrárias e Veterinárias – UNESP, Câmpus de Jaboticabal, como parte das exigências para a obtenção do título de Doutor em Agronomia (Genética e Melhoramento de Plantas).

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Orientador: Gustavo Vitti Mõro

Banca examinadora: Sandra Helena Unêda-Trevisoli, Rinaldo César de Paula, Maria Elisa Ayres Guidetti Zagatto Paterniani, João Antonio da Costa Andrade

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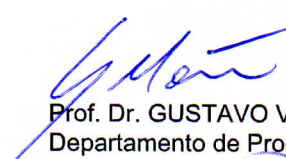
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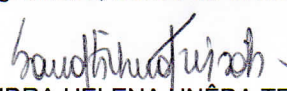
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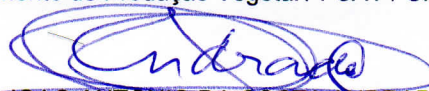
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Prof. Dr. GUSTAVO VITTI MÔRO
Departamento de Produção Vegetal / FCAV / UNESP - Jaboticabal


Prof. Dr. MARIA ELISA AYRES GUIDETTI ZAGATTO PATERNIANI
Secretaria de Agricultura, Centro de Grãos e Fibras - IAC / INSTITUTO AGRONÔMICO DE CAMPINAS


Profa. Dra. SANDRA HELENA UNÊDA TREVISOLI
Departamento de Produção Vegetal / FCAV / UNESP - Jaboticabal


Prof. Dr. JOAO ANTONIO DA COSTA ANDRADE
Departamento de Biologia e Zootecnica / Faculdade de Engenharia de Ilha Solteira


Prof. Dr. RINALDO CESAR DE PAULA
Departamento de Produção Vegetal / FCAV / UNESP - Jaboticabal

Jaboticabal, 22 de março de 2018

DADOS CURRICULARES DO AUTOR

CAMILA BAPTISTA DO AMARAL - filha de Rosângela Aparecida Baptista do Amaral e Milton José do Amaral e irmã de Lucas Baptista do Amaral, nascida aos 25 de janeiro de 1988, natural de Jaboticabal, interior do estado de São Paulo, Brasil. Coursou o ensino fundamental nas Escolas Estaduais “Antônio José Pedroso” e “Aurélio Arrobas Martins”. Concluiu o ensino médio juntamente com o ensino técnico profissionalizante no Colégio Técnico Agrícola “José Bonifácio” da Universidade Estadual Paulista - UNESP de Jaboticabal obtendo o título de Técnico em Agropecuária aos 12 de dezembro de 2005. Atuou como auxiliar de pesquisa na GRAVENA SGS em 2006/2007. Em fevereiro de 2007 ingressou no curso de Agronomia na UNESP – Faculdade de Ciências Agrárias e Veterinárias de Jaboticabal (FCAV). Como aluna de graduação foi professora voluntária no Cursinho Ativo, ministrando aulas de inglês. Foi Diretora Administrativa e Diretora Financeira da CAP Jr – empresa júnior da FCAV. Foi integrante do GIEU – Grupo de Integração Empresa-Universidade, participando da organização de diversos cursos. Estagiou nas áreas de Topografia, Produção Vegetal e Economia Rural. Obteve o título de Engenheira Agrônoma em fevereiro de 2012. Ingressou no curso de Pós-graduação em Agronomia (Produção Vegetal), nível de Mestrado, em agosto de 2012, pela Universidade Estadual Paulista UNESP – Faculdade de Ciências Agrárias e Veterinárias de Jaboticabal (FCAV), sendo bolsista CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior), tendo com orientador o Professor Dr. Leandro Borges Lemos, obtendo o título em julho de 2014, trabalhando com sistemas conservacionistas de produção. Ingressou no curso de Pós-graduação em Agronomia (Genética e Melhoramento de Plantas), nível de Doutorado, pela Universidade Estadual Paulista UNESP – Faculdade de Ciências Agrárias e Veterinárias de Jaboticabal (FCAV), sendo bolsista CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior), tendo com orientador o Professor Dr. Gustavo Vitti Mouro. É associada no Rotaract Club de Jaboticabal desde outubro de 2014, e associada ao Rotary Club de Jaboticabal desde outubro de 2017, realizando trabalho voluntário na comunidade.

“Pouco conhecimento faz com que as pessoas se sintam orgulhosas. Muito conhecimento, que se sintam humildes. É assim que as espigas sem grãos erguem desdenhosamente a cabeça para o Céu, enquanto que as cheias as baixam para a terra.”

Leonardo da Vinci

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CARACTERIZAÇÃO DE SINTÉTICOS DE MILHO QUANTO À EFICIÊNCIA DE USO DO NITROGÊNIO

RESUMO – Altas doses de nitrogênio são necessárias para atingir elevadas produtividades na cultura do milho, e o uso de genótipos eficientes no uso de nitrogênio permitiria atingir tais produtividades utilizando menor quantidade de fertilizantes nitrogenados. O objetivo do trabalho foi caracterizar sintéticos de milho quanto à eficiência de uso de nitrogênio e estudar as correlações entre os componentes da eficiência. Para caracterizar os sintéticos a campo, foram conduzidos experimentos na safra 2015/2016 e 2016/2017. Doze sintéticos e duas testemunhas comerciais (AL Avaré e Ipanema) foram cultivadas sob alta e baixa disponibilidade de nitrogênio em cada uma das safras, totalizando quatro experimentos. Sob baixa disponibilidade de nitrogênio, apenas 24 kg ha⁻¹ do elemento foram aplicados na semeadura, enquanto que sob alta disponibilidade, a adubação nitrogenada foi constituída de 24 kg ha⁻¹ na semeadura e 120 kg ha⁻¹ em cobertura no estádio V₄₋₅. Os experimentos foram conduzidos no delineamento de blocos ao acaso, e foram avaliados o índice do conteúdo de clorofila, acamamento, prolificidade e produtividade de grãos. A disponibilidade de nitrogênio alterou o índice do conteúdo de clorofila dos sintéticos, sendo que maiores valores foram observados sob alta disponibilidade de nitrogênio. Todas os sintéticos foram considerados adequados para cultivo sob baixa disponibilidade de nitrogênio, pois não houve diferença entre alta e baixa disponibilidade para os caracteres acamamento, prolificidade e produtividade de grãos. Considerando todos os caracteres estudados, dois sintéticos foram identificados como superiores, por apresentarem altos valores de índice de conteúdo de clorofila, resistência ao acamamento, prolificidade e produtividade de grãos, com desempenho superior às testemunhas. A fim de estudar os caracteres relacionados com a eficiência de uso de nitrogênio, dois sintéticos e uma testemunha, AL Avaré, foram cultivados em vasos, sob níveis contrastantes de disponibilidade de nitrogênio. Sob baixa disponibilidade, foram aplicados 100 mg dm⁻³ de nitrogênio em cada vaso na semeadura, e sob alta disponibilidade, foram aplicados 100 mg dm⁻³ por vaso na semeadura, 150 mg dm⁻³ em V₃ e 150 mg dm⁻³ em V₅. O experimento foi conduzido no delineamento de blocos ao acaso, em esquema fatorial 3x2. Foram avaliados o índice do conteúdo de clorofila, massa seca da parte aérea, massa seca da raiz, eficiência de absorção, eficiência de utilização e eficiência de uso de nitrogênio. O índice de conteúdo de clorofila diferiu entre os níveis contrastantes de nitrogênio, mas não entre os sintéticos. A massa seca da parte aérea foi diminuída pela baixa disponibilidade de nitrogênio, enquanto a resposta dos sintéticos para massa seca da raiz foi variável em função da disponibilidade de nitrogênio. Houve correlação forte e positiva entre eficiência de absorção e eficiência de uso de nitrogênio, sendo que a eficiência de absorção foi correlacionada com a massa seca das raízes.

Palavras-chave: eficiência de absorção, eficiência de uso, estresse abiótico, produtividade, *Zea mays*

CHARACTERIZATION OF MAIZE SYNTHETICS ON NITROGEN USE EFFICIENCY

ABSTRACT – High doses of nitrogen are necessary to reach high yields in maize crop, and the use of genotypes efficient in the use of nitrogen would allow to reach such productivities using less amount of nitrogen fertilizers. The objective of this work was to characterize maize synthetics as to nitrogen use efficiency and to study the correlations between the efficiency components. To characterize the synthetic in a field level, experiments were conducted in 2015/2016 and 2016/2017 seasons. Twelve synthetics and two commercial control (AL Avaré and Ipanema) were cultivated under high and low nitrogen availability in each of the seasons, totalizing four experiments. Under low nitrogen availability, only 24 kg ha⁻¹ of the element were applied at sowing, while under high availability, nitrogen fertilization consisted of 24 kg ha⁻¹ at sowing and 120 kg ha⁻¹ topdressed at the V4-5. The experiments were conducted in a randomized complete block design, and the chlorophyll content index, lodging, prolificacy and grain yield were evaluated. The nitrogen availability altered the chlorophyll content index of the synthetic, and higher values were observed under high nitrogen availability. All the synthetics were considered suitable for cultivation under low nitrogen availability, since there was no difference between high and low availability for lodging, prolificacy and grain yield. Considering all the traits studied, two synthetics were identified as superior, because they presented high values of chlorophyll content index, lodging resistance, prolificacy and grain yield, with superior performance to the control. In order to study the traits related to nitrogen use efficiency, two synthetics and one control, AL Avaré, were grown in pots, under contrasting levels of nitrogen availability. Under low availability, 100 mg dm⁻³ of nitrogen was applied to each pot at sowing, and under high availability, 100 mg dm⁻³ per pot was applied at sowing, 150 mg dm⁻³ at V₃ and 150 mg dm⁻³ at V₅. The experiment was conducted in a randomized complete block design, in a 3x2 factorial scheme. The chlorophyll content index, shoot dry mass, root dry mass, uptake efficiency, utilization efficiency and nitrogen use efficiency were evaluated. The chlorophyll content index differed between the contrasting levels of nitrogen, but not among the synthetics. The shoot dry mass was diminished by the low nitrogen availability, while the response of the synthetics to root dry mass was variable as a function of the nitrogen availability. There was a strong and positive correlation between uptake efficiency and nitrogen use efficiency, and the uptake efficiency was correlated with the root dry mass.

Keywords: uptake efficiency, use efficiency, abiotic stress, grain yield, *Zea mays*

CAPÍTULO 1 – Considerações Gerais

1. INTRODUÇÃO

Produzir alimentos em quantidade suficiente para atender a demanda da população de maneira sustentável é um dos desafios para os próximos anos, principalmente porque os recursos naturais disponíveis devem ser preservados. Estima-se que em 2050, a população mundial irá atingir 9,6 bilhões de pessoas, e que em 2100 esse valor ultrapasse 10,5 bilhões (GERLAND et al., 2014). Afim de assegurar que a demanda por alimentos e biocombustíveis seja suprida, a produção agrícola precisa, no mínimo, ser dobrada até 2050 (RAY et al., 2013) e, neste contexto, a cultura do milho (*Zea mays* L.) tem grande importância, uma vez que é responsável, em média, por 32% do total de calorias consumidas no mundo (SHIFERAW et al., 2011).

A produtividade da cultura do milho no mundo tem aumentado em 1,6% ao ano, o que resulta em aumento de 67% até 2050. Todavia, as projeções indicam que este aumento anual deveria ser da ordem de 2,4% para que a produção agrícola possa dobrar até 2050 (RAY et al., 2013). Uma das razões para o baixo incremento de produtividade na cultura é a baixa disponibilidade de nutrientes, que juntamente com outros fatores, pode resultar em perdas globais de produtividade de até 9.000 kg ha⁻¹ (LOBELL; CASSMAN; FIELD, 2009). O nitrogênio (N) se destaca neste cenário por ser o nutriente requerido em maior quantidade pela cultura do milho, sendo que o valor estimado de extração deste elemento pode chegar a 361 kg ha⁻¹ de N para atingir uma produtividade de grãos da ordem de 13.000 kg ha⁻¹ (SILVA et al., 2015).

A grande quantidade de N requerida pela cultura do milho torna-se problema uma vez que em regiões desenvolvidas, o uso intensivo de fertilizantes nitrogenados acarreta em custo energético e econômico, além de ser potencialmente poluidor (MISHIMA; TAKADA; KITAGAWA, 2011). Por sua vez, em regiões que estão em desenvolvimento, a baixa disponibilidade natural deste elemento no solo é uma das principais causas da baixa produtividade (HU et al., 2008), o que aliada ao elevado custo dos fertilizantes nitrogenados, pode tornar a produção inviável. Desta forma, o desenvolvimento de cultivares eficientes no uso do N é de fundamental importância

para o aumento da produtividade com sustentabilidade, uma vez que permitiria a diminuição do uso dos fertilizantes nitrogenados e a obtenção de maiores produtividades sob baixa disponibilidade de N.

Para que o melhoramento de plantas seja eficiente é necessário que, entre outros fatores, haja variabilidade genética. Por isso, os sintéticos constituem uma importante fonte de variabilidade na busca por genes de tolerância ou resistência aos fatores abióticos (CANCELLIER et al., 2012), uma vez que, apesar da baixa produtividade, possuem complexa estrutura genética (TALABI, BADU-APRAKU; FAKOREDE, 2017).

Diante do exposto, o objetivo do presente trabalho foi caracterizar sintéticos de milho quanto à eficiência de uso de N e estudar as correlações entre os componentes da eficiência.

2. REVISÃO DE LITERATURA

2.1. Por que milho?

A cultura do milho é de extrema importância no cenário mundial por seu valor nutricional e uso na alimentação humana direta, indireta e aplicações industriais (GARCIA et al., 2006), sendo a terceira cultura em área cultivada e a primeira em produção e produtividade. Segundo levantamento feito pelo USDA - *United States Department of Agriculture*, no ano agrícola 2015/2016 foram produzidas 961 milhões de toneladas de milho no mundo, das quais 36% foram produzidos pelos Estados Unidos, seguido pela China (23%) e pelo Brasil (7%) (USDA, 2017). Para o ano agrícola 2016/2017, estima-se que o total de grãos produzidos seja de mais de um bilhão de toneladas. No Brasil, a produção em 2015/2016 foi de 67 milhões de toneladas no total, sendo 39% na primeira safra e 61% produzidos na segunda safra (CONAB, 2017).

Apesar de figurar entre os maiores produtores, o Brasil está muito aquém no que diz respeito à produtividade. Em 2015/2016, a produtividade média nacional foi de 4.799 kg ha⁻¹ na primeira safra e 3.904 kg ha⁻¹ na segunda safra (CONAB, 2017), contra 10.570 kg ha⁻¹ nos Estados Unidos e 10.330 kg ha⁻¹ no Canadá, cuja produção na mesma safra foi de apenas 20% do montante brasileiro. Assis et al. (2006), ao estimarem a produtividade potencial da cultura do milho de acordo diversas condições climáticas, observaram que o potencial produtivo mínimo está entre 12.000 kg ha⁻¹ e 17.000 kg ha⁻¹ para híbridos, e o máximo está entre 13.000 kg ha⁻¹ e 19.000 kg ha⁻¹. Tais dados corroboram a ideia de que o potencial produtivo dos cultivares não está sendo expresso, e, no caso do Brasil, essa perda potencial é de mais de 50%.

A explicação para a baixa produtividade real frente ao potencial dos cultivares modernos está no fato de que esta expressão, dada pelo fenótipo, é a soma do efeito do genótipo e do ambiente e, no contexto de ambiente, um dos principais fatores que afeta a produtividade das culturas, em especial do milho, é a baixa disponibilidade de nutrientes (LOBELL; CASSMAN; FIELD, 2009).

2.2. Por que nitrogênio?

O nitrogênio (N) é o elemento requerido em maior quantidade pela maioria das culturas agrícolas (MALAVOLTA; VITTI; OLIVEIRA, 1997), sendo classificado como macronutriente primário; tem função estrutural nas plantas, participando da estrutura de aminoácidos, ácidos nucleicos, flavonoides e da clorofila (BUCHANAN et al., 2000). Devido à importância para as culturas agrícolas, o N é o nutriente aplicado em maior quantidade na agricultura, o que também faz com que as perdas deste elemento sejam elevadas. Segundo Conant, Berdanier e Grace (2013), aproximadamente 60% do N aplicado via fertilizantes é perdido ao invés de ser absorvido pelas culturas.

Devido à participação direta deste elemento nas proteínas, o acúmulo de N possui correlação direta com a produção de fitomassa e, conseqüentemente, com a produtividade das culturas. Neste sentido, Fernandes, Arf e Andrade (2005) estudaram a aplicação de doses de até 180 kg ha⁻¹ de N nos híbridos AG 9010, CO32, XB8010, DKB333 B e variedades BR 106 e Sol da Manhã, e verificaram que a máxima produtividade foi alcançada sob a dose estimada de 110 kg ha⁻¹ de N. Gava et al. (2010) obtiveram aumento de produtividade até a dose de 200 kg ha⁻¹ de N para o híbrido AG5011. Segundo Aguiar et al. (2014), a recomendação de adubação para obtenção de 6.000 a 8.000 kg ha⁻¹ de grãos é de, no máximo, 120 kg ha⁻¹ de N.

Queiroz et al. (2011) observaram incremento linear na produtividade de grãos de milho híbrido RB9308 YG sob doses de até 160 kg ha⁻¹ de N, e que o incremento entre a não aplicação de N e a maior dose foi de 1.773 kg ha⁻¹ de grãos. Silva et al. (2015) obtiveram produtividades em torno de 10.000 kg ha⁻¹ ao avaliarem os híbridos AG30A86, AGN20A55 e CD308, com extração de N variando entre 283 e 314 kg ha⁻¹. Li et al. (2017), ao estudarem o efeito do N sobre o híbrido Zhengdan 958, detectaram aumento significativo de produtividade em função da aplicação de doses de N até 315 kg ha⁻¹. Todos esses trabalhos evidenciam a importância do nitrogênio para a obtenção de altas produtividades independentemente do material genético. Isso ocorre porque o melhoramento genético geralmente é feito sob altas doses de N para permitir a máxima expressão do potencial produtivo, o que garante genótipos responsivos, mas não necessariamente eficientes (MARTINS et al., 2008).

A importância do N não se restringe apenas à produtividade de grãos, tendo grande influência sobre o desenvolvimento radicular. Trachsel et al. (2013) avaliaram 108 linhagens de milho, divididas em grupos com sistema radicular com desenvolvimento relativamente horizontal (raso) ou vertical (íngreme) sob alta e baixa disponibilidade de N, e observaram que sob baixa disponibilidade de N, as raízes das linhagens de sistema radicular raso tornaram-se íngremes, indicando que esta condição favorece o aprofundamento das raízes. Postma, Dathe e Lynch (2014) estudaram o desenvolvimento de raízes laterais em milho e concluíram que a densidade e comprimento deste tipo de raiz depende da disponibilidade relativa de N no solo, sendo que quando este elemento, juntamente com fósforo e fixação de carbono estão presentes, a densidade de raízes laterais é otimizada para garantir a absorção dos nutrientes.

2.3. Por que eficiência de uso do nitrogênio?

O termo eficiência pode ser atribuído a diversas definições. Uma das mais utilizadas no âmbito da eficiência de uso do N (EUN) é a descrita por Moll, Kamprath e Jackson (1982), que define EUN como sendo a relação entre a massa de grãos produzida e a quantidade de N disponível no solo. A eficiência não deve ser confundida com tolerância, que representa a capacidade do genótipo em resistir ao estresse (DO VALE et al., 2011). Miti, Tongoona e Derera (2010) definiram que tolerância é representada pela diferença entre a produtividade sob condições ideais e sob condições de estresse, sendo que os genótipos mais tolerantes são aqueles que apresentam menor diferença entre as condições.

A seleção de indivíduos superiores nos programas de melhoramento geralmente é feita em ambientes com alta disponibilidade de N, mas que vez sob baixa disponibilidade, a variância genotípica, herdabilidade e acurácia seletiva tendem a ser menores (GUEDES et al., 2015). Como consequência, para expressar seu potencial produtivo, os genótipos selecionados necessitam de altas doses de N (CARLONE; RUSSEL, 1987; MARTINS et al., 2008), e não são adequados para o cultivo em ambientes com baixa disponibilidade deste elemento. Este fato se deve à expressão diferencial de genes entre os ambientes contrastante para N.

Ribault et al. (2007) caracterizaram QTL (*quantitative trait loci*) em uma população segregante de milho cultivada sob condições contrastantes de disponibilidade de N. Os autores encontraram três QTL para produtividade de grãos sob alta disponibilidade de N e de cinco a oito QTL sob baixa disponibilidade, sendo que apenas uma das regiões identificadas foi comum às duas condições de disponibilidade de N. Desta forma, a seleção de genótipos visando ambientes com baixo N é mais eficiente quando feita sob essas condições (BÄZINGER; LAFITTE, 1997). Tal pressuposto pode ser confirmado observando-se os resultados obtidos por Al Naggari et al. (2017), que avaliaram o ganho de seleção em trigo tolerante à baixo N selecionado em ambiente com alta e baixa disponibilidade de N, e obtiveram ganhos de 5,11 e 7,70% em alto N e 19,40 e 23,14% sob baixo N para produtividade por planta.

Apesar da seleção de genótipos sob alta disponibilidade de N não ser adequada para cultivo sob baixa disponibilidade, o contrário não necessariamente é verdadeiro, uma vez que já foram identificados QTLs contendo genes de efeito aditivo que são estáveis entre as disponibilidades de N, incluindo regiões que indicam independência parcial do controle da produtividade de grãos frente a disponibilidade de N (RIBAULT et al., 2007). Desta forma, levando em consideração que os mecanismos genéticos que controlam a produtividade sob baixa disponibilidade de N são mais complexos do que sob alta disponibilidade, faz sentido inferir que a chance de selecionar genótipos com “dupla aptidão” é maior quando a seleção é feita com foco em eficiência sob baixo N.

O melhoramento visando a eficiência de uso de N é fundamental para permitir o aumento da produção com sustentabilidade. O N-inorgânico utilizado na adubação, quando volatilizado, causa efeitos negativos na atmosfera, enquanto o N-lixiviado, quando atinge lençóis freáticos, pode causar a eutrofização de cursos d'água (MARTINELLI, 2007). Brackin et al. (2015) estudaram a capacidade de absorção de raízes de cana-de-açúcar e observaram que o fluxo de nitrato proveniente da adubação via ureia ultrapassou em 527% a capacidade máxima de absorção de N por unidade de superfície de raiz, evidenciando que grande parte do N aplicado é possivelmente perdido e pode levar à contaminação, especialmente nos países desenvolvidos, onde as doses de N aplicadas são altas (MISHIMA; TAKADA;

KITAGAWA, 2011). Por outro lado, nos países em desenvolvimento, a quantidade de N utilizada pelos produtores não é suficiente para atingir elevadas produtividades (HU et al., 2008), e por isso, a eficiência de uso de N é importante em ambos os casos, permitindo a obtenção de altas produtividades com sustentabilidade.

2.4. Por que sintéticos?

Para que o melhoramento de plantas seja possível, é necessário que, entre outros fatores, haja variabilidade genética. Por isso, os sintéticos constituem uma importante fonte de variabilidade na busca por genes de tolerância ou resistência aos fatores abióticos (CANCELLIER et al., 2012), uma vez que, apesar da baixa produtividade relativa, possuem complexa estrutura genética (SHULL, 1908; ANDRADE; MIRANDA FILHO, 2008), apresentando grande variabilidade entre e dentro das populações (SEMAGN et al., 2014).

Além de servir como fonte de germoplasma, esse tipo de genótipo pode dar origem a variedades de polinização aberta, que constituem alternativa para produtores pouco tecnificados, uma vez que são mais produtivas que variedades crioulas e geralmente mais estáveis que os híbridos (SETIMELA et al., 2007). Além disso, o uso das sementes da safra anterior resulta em perda de 5% de produtividade no caso das variedades, enquanto que nos híbridos, essa perda é estimada em 32% (PIXLEY; BÄZINGER, 2004), o que viabiliza a produção em pequenas propriedades sem a necessidade de aquisição de sementes. Estima-se que no Brasil, as variedades de polinização aberta representem pouco mais que 7% dos genótipos de milho cultivados (GALVÃO et al., 2015), enquanto que em países africanos, este percentual pode chegar a 100% (KASSIE et al., 2012).

Poucos são os trabalhos relatando programas de melhoramento específicos para variedades, visto que na maioria dos programas, as variedades são utilizadas como fonte de germoplasma visando produção de híbridos ou como um desdobramento dos programas de melhoramento de híbridos. O CYMMIT – Centro Internacional de Melhoramento de Milho e Trigo, divulgou recentemente dados do programa de melhoramento de variedades para tolerância aos estresses abióticos na África. As variedades, obtidas a partir de linhagens-elite selecionadas dentro de um mesmo grupo heterótico, apresentaram produtividade de grãos entre 1.600 a 4.290 kg

ha⁻¹ para condições de déficit hídrico, entre 1.870 e 6.040 kg ha⁻¹ em condições de baixo N e entre 4.310 e 9.350 kg ha⁻¹ em condições ótimas, em uma população de plantas de 66.667 plantas ha⁻¹ (MASUKA et al., 2017).

No Brasil, as variedades de polinização aberta são produzidas, em sua maioria, por empresas estatais e estaduais, mas os programas geralmente não contemplam condições de estresses abióticos. Emygdio et al. (2015), avaliando o desempenho de variedades desenvolvidas pela Embrapa – Empresa Brasileira de Pesquisa Agropecuária, obtiveram produtividades entre 4.683 e 11.103 kg ha⁻¹ sob condições ótimas de cultivo. Outras instituições que possuem variedades em seu catálogo de cultivares são a Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina, a Coordenadoria de Assistência Técnica Integral, detentora da variedade AL Avaré, e a empresa DiSolo, detentora da variedade Ipanema.

Os sintéticos utilizados neste trabalho foram por meio do Método Irlandês, a partir da separação de linhagens elite em grupos de acordo com a origem e tempo de maturação fisiológica. Posteriormente, as linhagens foram cruzadas em *sib* dentro dos grupos e, na safra seguinte, recombinadas utilizando-se as progênies como parental feminino e uma mistura de todas as progênies do grupo como parental masculino (Di Salvo, 2011). Os sintéticos obtidas apresentaram, em sua maior parte, características de grão duro ou semiduro, e coloração amarela.

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CAPÍTULO 2 – Phenotyping synthetic maize populations for environments with low nitrogen availability

Abstract – A major constraint in maize production is the high nitrogen requirement of this crop since the soil of several countries have low availability of this component. A 2-year field experiment was conducted to characterize synthetic maize populations by identifying possible sources of variability targeting low N environments. Twelve synthetic maize populations and two checks, AL Avaré and Ipanema, were cultivated under high and low nitrogen levels and evaluated for chlorophyll content index, lodging, prolificacy and grain yield. The results indicate that the nitrogen level affected the chlorophyll content index only, showing that the applied nitrogen was sufficient to express differences for this trait between the two studied environments. In addition, nitrogen level affected the percentage of lodged plants of two synthetics only. Grain yield and lodging were adequate to discriminate the synthetics. Among the studied synthetic maize populations, 17% considered as superior due their performance for lodging-resistance and grain yield.

Keywords: *Zea mays* L; plant breeding; gt biplot; abiotic stress.

1. Introduction

Nitrogen (N) is the major nutrient taken up by maize (*Zea mays* L.) and the main constituent of chlorophyll (WASSAYA et al., 2017) as well. For this reason, nitrogen plays a key role in important agronomic traits, such as lodging, grain yield, and prolificacy. Shi et al. (2016) reported that increased stalk resistance under adequate N supply, resulted in less lodged plants. Akhtar et al. (2015) studied the nitrogen fertilization effect on maize and concluded that the nutrient had significant effect on increasing grain yield. Prolificacy, defined as ears produced per plant, is an important secondary trait for breeding under low N conditions (TALABI et al., 2017) since it results in higher grain yield.

Usually, high N environments are used in experiments to select superior maize cultivars, leading to genotypes with high yield under these conditions, but not

necessarily efficient under low N environments (BADU-APRAKU et al., 2012). However, sub-optimal soil nitrogen availability constitutes an important restriction to crop production in several countries (HU et al., 2008), which makes developing cultivars adapted to low N condition a necessity for maize breeding programs.

The best methodology to select genotypes for low N environments is to compare the performance of these genotypes in environments with high and low N availability (HAN et al., 2015), to identify genotypes carrying genes associated with tolerance to low N that can be used in breeding programs to obtain superior and N efficient cultivars.

The development of superior cultivars depends on accurate characterization of the genotypes, which is only possible in the presence of variability. Synthetic maize populations are less productive than modern cultivars such as hybrids, however, its complex genetic structure makes them an important source of genes related to tolerance to abiotic stress (COIMBRA et al., 2010). In addition, N use is significantly different among maize synthetics (ZHANG et al., 2007; ASARE et al., 2011), and an accurate phenotyping is necessary to identify those with potential to be used as source of variability in breeding programs aiming at low N conditions. Phenotyping is the identification of the phenotype as a result of genotypic and environmental differences, and efficiency in phenotyping is a major constraint to obtain genetic gain in breeding programs (ARAUS et al., 2018).

The objective of this study was to characterize synthetic maize populations and identifying possible sources of variability targeting low N environments.

2. Material and methods

2.1 Experimental site

The experiments were conducted in Jaboticabal, SP, Brazil (21°14'33"S, 48°17'10"W, and 565 m above sea level), in the first seasons of 2015/2016 and 2016/2017. The experimental soil is characterized as eutroferric red Oxisol in both areas. The regional climate is classified as Aw type (savanna with dry winter) according to Köppen. The area was maintained under traditional cultivation system, and maize was sowed in the last three years.

Before the experiments were implemented, soil samples were collected in 10 points per experimental area in the 0-20 cm layer and analysis were performed according to Raij and Quaggio (1983). The results for both harvests are shown in Table 1, as well as the accumulated rainfall in the periods.

Table 1. Results from the soil analyses of layer 0-20 cm, performed before the installation of the experiment, and accumulated rainfall during the conduction of the experiment in Jaboticabal, SP.

Soil parameters	2015/2016	2016/2017
pH (CaCl ₂)	6.3	5.5
Organic matter (g dm ⁻³)	25.5	25.0
P (resin) (mg dm ⁻³)	32.0	35.0
S (mg dm ⁻³)	5.5	12.0
H + Al (mmol _c dm ⁻³)	24.5	15.0
K (mmol _c dm ⁻³)	4.7	3.1
Ca (mmol _c dm ⁻³)	43.0	36.0
Mg (mmol _c dm ⁻³)	15.5	15.0
CTC (mmol _c dm ⁻³)	97.7	69.3
V (%)	73	78
Accumulated rainfall		
From sowing to top-dressing (mm)	387.4	127.2
From top-dressing to flowering (mm)	479.9	261.8
From flowering to physiological maturation (mm)	357.3	301.6
Total rainfall (mm)	1,224.6	690.6

2.2. Treatments and experimental design

Twelve synthetics maize populations and two checks were evaluated in this study. The synthetics were obtained by the recombination of elite lines, following the procedure described by Oliveira et al. (2016) whereas the checks were the Ipanema and AL Avaré commercial open-pollinated varieties. AL Avaré features high resistance

to lodging and high yield potential, while Ipanema is known by having a high developed root system.

In each season, the two different experiments consisted of cultivating the 14 maize genotypes in two soils with contrasting N availability, low and high. All experiments were fertilized with 24 kg ha⁻¹ N, 84 kg ha⁻¹ P₂O₅ and 48 kg ha⁻¹ K₂O at sowing, using the formula 08-28-16 as source. In addition, the high N experiments were top-dressed with 120 kg ha⁻¹ of N via urea, applied continuously next to the seeding line during the growth stage of 4-5 fully expanded leaves. The 2016/2017 experiment also received 25 kg ha⁻¹ KCl due to low potassium level, besides top-dressed urea.

The experiments followed a randomized complete block design, with five replicates. Each plot consisted of two 5 m-long rows, 0.5 m apart and 36 cm between plants in the row, totaling 28 plants per plot. The 2015/2016 experiment was sowed on November 18, 2015, and harvested on April 15, 2016, while the 2016/2017 experiment was sowed on November 23, 2016, and harvested on March 27, 2017. Supplementary irrigation was not needed.

2.3. Evaluated traits and statistical analyses

At flowering, chlorophyll content index was measured at the middle third of the leaf above the ear using the CCM-200-Opti-science equipment. At maturity, lodging (broken below the ear or leaning more than 45 degrees from the vertical) was recorded for each plot and expressed as percentage of the total number of plants in the plot. After physiological maturity, the ears of both rows of the plot were hand-harvested; the kernels were separated and weighed to determine moisture content. The grain yield was adjusted to 13% grain moisture and corrected by covariance of ideal stand for 56.000 plants ha⁻¹. Prolificacy was given by the ratio between total number of ears per plot and number of plants per plot.

Data were tested for normality using the R software (R COR TEAM, 2014), and no transformation was needed. Individual analysis of variance was carried out for each experiment separately to verify the uniformity of residual variance, followed by joint analysis of variance, considering N availability and synthetic as fixed effects. Tukey

test was used to compare the means, and t test was used to compare the individual performance of the synthetics between N levels. All these statistical analyses were performed using the PROC GLM procedure from SAS™ version 9.2 (SAS INSTITUTE INC, 2009).

The characterization of the synthetics was made using a multivariate approach to determinate the “ranking genotypes”, considering all traits in a genotype-by-trait (GT) biplot model, using standardized values of the traits. The traits were considered as the tester and the cultivars as entries. For lodging, it was used the percentage of non-lodged plant, allowing the selection of synthetics with the highest value for all traits. This analysis used the model described by Paramesh et al. (2016). The GT Biplot was constructed using the package “GGEBiplotGUI”, test-centered and based on genotype metric preserving (row metric preserving).

3. Results

3.1. Analysis of variance and means comparison

The coefficient of determination (R^2), for chlorophyll content index, lodging, prolificacy and grain yield was higher than 0.80 (Table 2).

Table 2. Summary of the joint analyses of variance for chlorophyll content index (CCI), lodging (LOD), prolificacy (PROL) and grain yield (GY) of 12 synthetic maize populations and 2 checks in two seasons, under high and low nitrogen availability.

Source	DF	Mean squares			
		CCI	LOD	PROL	GY
Nitrogen (N)	1	156.11*	12.01 ^{ns}	0.0476 ^{ns}	341252 ^{ns}
Genotype (G)	13	347.39**	202.08**	0.147**	12543587**
N x G	13	22.11 ^{ns}	31.56 ^{ns}	0.012 ^{ns}	278166 ^{ns}
Error	224	36.74	23.66	0.016	529568
R ²	-	0.90	0.89	0.90	0.96

**, * e ^{ns}: significant at 1%, 5% and non-significant by the F test, respectively. R²: coefficient of determination.

Nitrogen level significantly influenced the chlorophyll content index at flowering but had no effect on the other traits. The different synthetics had significant effect for all trait, but there was no interaction between synthetics and N levels (Table 2).

Chlorophyll content index at flowering was 2% higher for high N level compared to low N. The chlorophyll content index ranged from 50.48 to 67.15 among the studied synthetics. Tukey test differentiated four synthetics with significant higher chlorophyll index values, including check Ipanema, above 61.70, while five synthetics had indices lower than 56.73. The synthetics with the lowest values included the AL Avaré check (Table 3).

Table 3. Means of chlorophyll content index (CCI), lodging (LOD, %), prolificacy (PROL, n^o) and grain yield (GY, kg ha⁻¹) of 12 synthetic maize populations and 2 checks in two years, under high (HN) and low (LN) nitrogen availability.

		CCI	LOD	PROL	GY
Nitrogen	LN	57.58	4.68	1.00	5,598
	HN	59.08	5.10	1.03	5,668
Genotype	A	57.22 bc ¹	2.05 e	1.03 bc	6,297 a
	B	54.22 cd	1.85 e	1.04 bc	6,194 a
	C	56.73 bcd	3.80 cde	1.05 bc	6,224 a
	D	57.20 bc	1.70 e	0.96 bc	6,587 a
	E	58.17 bc	2.30 de	0.97 bc	6,570 a
	F	61.77 ab	9.50 ab	1.01 bc	5,014 cd
	G	58.68 bc	7.40 abcd	0.94 c	4,789 cd
	H	67.15 a	3.65 cde	1.00 bc	4,987 cd
	I	50.48 d	10.60 a	1.28 a	4,959 cd
	J	54.42 cd	3.30 cde	0.95 bc	4,373 d
	K	62.54 ab	8.15 abc	0.97 bc	4,982 cd
	L	60.18 bc	8.25 abc	0.96 bc	5,301 bc
	AL Avaré	56.15 bcd	1.80 e	1.08 b	6,681 a
	Ipanema	61.70 ab	4.35 bcde	1.00 bc	5,904 ab
Mean		58.33	4.89	1.02	5,633
Standard error		0.43	0.38	0.009	74.74

¹Means followed by the same letter did not differ based on Tukey's test.

Average lodging was 4.89% and did not affect by N level. Five synthetics populations had the highest percentage of lodged plants, ranging from 7 to 11%. The lowest lodging percentage, 2%, was observed for AL Avaré, which was similar to 50% of the synthetics and the other check, Ipanema (Table 3).

Similarly, N level did not affect prolificacy, and the plants produced, on average, one ear per plant for both N levels. Also, prolificacy ranged between 0.94 and 1.28 for synthetics G and I, respectively. The checks presented intermediate values of 1.08 and 1.00 for AL Avaré and Ipanema, respectively (Table 3).

Also, nitrogen level did not affect grain yield, which was of 5,633 kg ha⁻¹, and the synthetics showed values ranging from 4,373 kg ha⁻¹ to 6,681 kg ha⁻¹. The checks and 42% of the synthetics had grain yield up to 5,904 kg ha⁻¹, whereas the other synthetics had values at least 603 kg ha⁻¹ lower (Table 3).

Overall, N level affected chlorophyll content index and did not affect prolificacy and grain yield of the studied synthetics; however, it affected lodging of some synthetics. The lodging percentage of synthetic K increased ($p < 0.05$) by 58% under low N, while synthetic L decreased ($p < 0.01$) by 49% in the same condition (Figure 1). The other synthetics were not affected by N level.

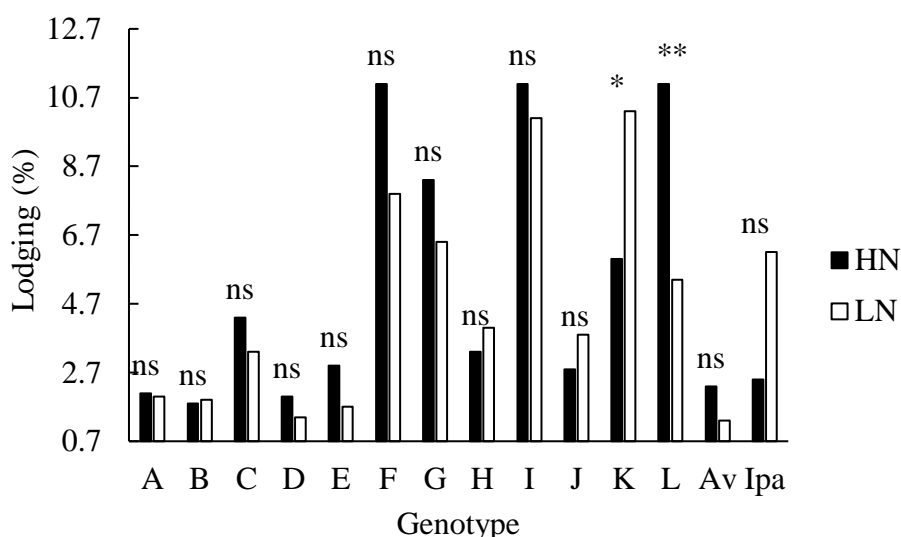


Figure 1. Means of lodged plants (%) of 12 synthetic maize populations and 2 checks under low level (LN) and high level (HN) of nitrogen. ** and*: significant at 1% and 5% by the F test, respectively. Av: AL Avaré. Ipa: Ipanema.

3.2. GT Biplot

The mean performance of chlorophyll content index, percentage of non-lodged plants, prolificacy and grain yield under low N levels were used to identify the synthetics with the best performance (Figure 2).

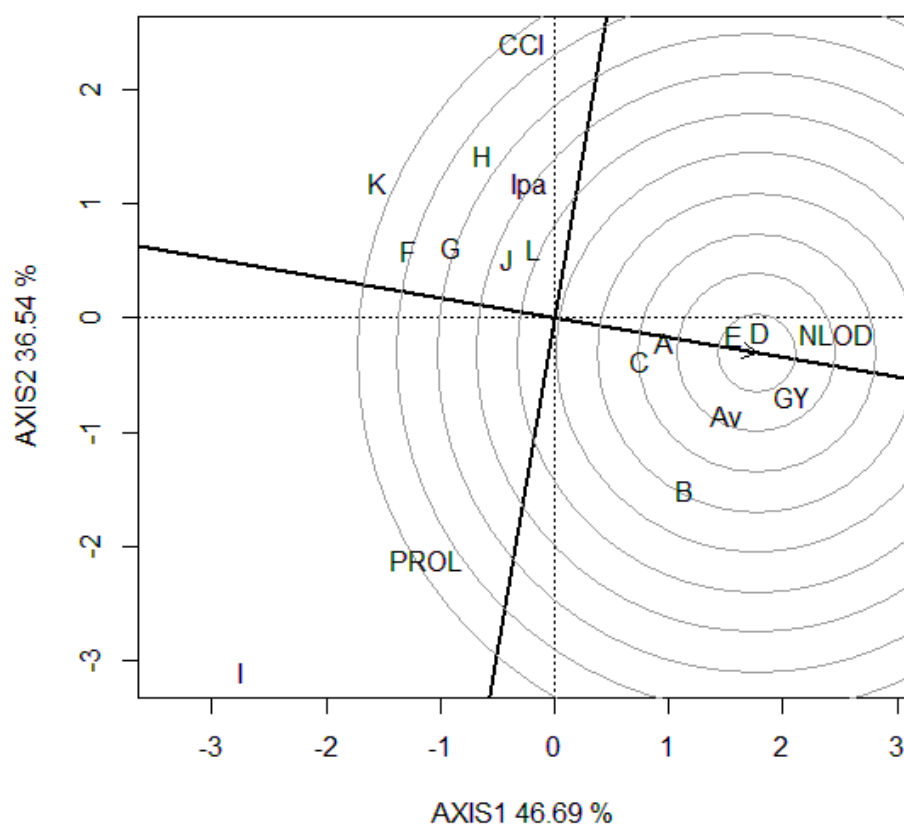


Figure 2. A vector view of genotype x trait biplot showing the ranking of 12 synthetic maize populations and 2 checks for chlorophyll content index (CCI), percentage of non-lodged plants (NLOD), prolificacy (PROL) and grain yield (GY) under low nitrogen availability. Av: AL Avaré. Ipa: Ipanema.

The principal components PC1 and PC2 described as AXIS1 and AXIS2, respectively, explained 83.23% of the total variation observed among the traits (Figure 2). The synthetics considered as ideal were D and E, allocated at the innermost concentric circle with an arrow, followed by the check AL Avaré. The check Ipanema was allocated in the seventh concentric circles. Among all studied synthetics, 58% are outside the concentric circles and considered as “non-ideal”. Grain yield and percentage of non-lodged plants were the traits that most affected the synthetics.

4. Discussion

R^2 values obtained indicate that variability is due to genotype, and not to environment, as well good experimental precision (CARGNELUTTI FILHO; STORCK, 2007) and a high probability of selection on genetic traits that may be heritable.

Nitrogen is an essential nutrient that plays a key role in the chlorophyll content, as part of the molecule itself and in its synthesis (WASAYA et al., 2017) and therefore, the chlorophyll index is expected to increase when N levels in the soil are high. Wei et al. (2016) evaluated the response of maize to field conditions with and without N fertilizers and reported that the leaf chlorophyll concentration decreased under low N compared to high N. To this end, the increase in chlorophyll content also indicates that the N dose applied on this study was able to distinguish the environments regarding this trait.

Because chlorophyll plays a major role in photosynthesis, higher values of chlorophyll index are often associated with higher grain yield. Széles et al. (2012) reported strong positive phenotypic correlation between chlorophyll content at flowering and grain yield, and between N levels and chlorophyll content. Al-Naggar et al. (2015) studied the effect of four increasing N doses on chlorophyll content index, also measured by CCM-200, and on grain yield per plant of 15 diallel maize crosses, and found values ranging from 28.9 to 56.4 for chlorophyll index and 87.8 to 163.8 g per plant for grain yield. Despite the effect of chlorophyll content index, the amplitude between N levels in this study was not sufficient to affect the other traits, including grain yield. Also, it is important to highlight that, despite the effect of chlorophyll content on plant performance, the genotype effect and the biological potential must be considered.

The synthetics studied showed difference for all traits (Table 2), and variability among quantitative traits of different maize genotypes have been widely reported (SERPOLAY-BESSON et al., 2014; YANG et al., 2016), and it is a prerequisite for the selection process.

Interaction between maize hybrids and N levels are shown in many studies, indicating that these genotypes are recommended for high or low N environments (HAN et al., 2015). However, Semagn et al. (2014) demonstrated that synthetics maize populations are constituted by a high number of genotypes leading to large variability

within the population, implying in high ability to maintain performance across multiple environments (MANSFIEL; MUMM, 2014). In this case, the complex structure of the synthetics confers the ability to maintain the average performance, regardless of N level, contrary to what happens for hybrids. Likewise, Ferro et al. (2007) studied maize landraces and observed no differences on individual means when N was applied to the crop, evidencing that populations constituted by a large number of genotypes are usually less responsive to environmental changes.

Nitrogen application can increase lodging-resistance, but this effect is not absolute for all genotypes. Shi et al. (2016) evaluated the effect of different N rates on maize genotypes and reported that N can improve the quality of the stalk, which leads to higher lodging-resistance, but this effect was observed only for the lodging-susceptible genotype. On the other hand, N application can increase plant height (CARPICI et al., 2010), which also increases lodging.

The differential performance for chlorophyll content index, lodging, prolificacy and grain yield indicates a significant difference between the studied synthetics. Usually, synthetics can be subjected by populational breeding methods to form open-pollinated varieties with increased frequency of favorable alleles maintaining the genetic variability (AVDIKOS et al., 2011), and the selection is made under high N conditions (BADU-APRAKU et al., 2012). Therefore, it is expected that the checks have a high frequency of favorable alleles, but the superior performance of the synthetics under low N suggests a higher frequency of these alleles.

The multivariate analysis showed that prolificacy and chlorophyll content index contributed less to discriminate the synthetics compared to percentage of non-lodged plants and grain yield. These results differ from what is mentioned in the literature, where prolificacy is considered as an important secondary trait to improve selection in maize breeding programs targeting low-N environments (BÄNZIGER; LAFITTE, 1997). The explanation is the reduced spacing between rows, that decreases the prolificacy in maize (MADDONNI; MARTÍNEZ-BERCOVICH, 2014).

The success of maize breeding depends on maintaining the genetic variability. Talabi et al. (2017) evaluated the genetic variance and predicted gain under cycles of selection of 250 maize progenies, concluding that the lack of genetic variability is leading to slow selection progress under low N, and that it is necessary to introgress

new genotypes with favorable alleles to ensure progress from selection. Thus, the synthetics identified as superior could be used as source of variability under low N conditions or as commercial cultivars indicated for areas with low N availability.

5. Conclusions

We conclude that grain yield and lodging are the most suitable traits to characterize the synthetics under low N level, since chlorophyll content index and prolificacy had little influence to explain the variability in the evaluated genotypes. Overall, nitrogen level had no effect on the individual performance of synthetics. All synthetics were considered adequate under low N environments since their performance is not affected by N availability, but only 17% were superior than checks due to increased percentage of non-lodged plants and grain yield. Therefore, these synthetics can be used as source of variability for extracting inbred lines or developing inter-varietal hybrids with increased chlorophyll content index, prolificacy and grain yield and low lodging-susceptibility, but further studies must be conducted to evaluate the effect of the genes associated with these traits, in order to determine the more adequate breeding strategy.

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CAPÍTULO 3 – Analysis of nitrogen use efficiency factors in early stages of maize

Abstract – Increasing the nitrogen (N) use efficiency in maize is important to ensure an increase in productivity. The objective of this study was to identify the traits related to N use efficiency in early stages of synthetics maize populations, based on the components of N use efficiency, dry mass accumulation, and chlorophyll content index. The chlorophyll content index was efficient for distinguishing the environments regarding the availability of N, but it was not adequate for discriminating the synthetics studied. The dry mass of the shoot was affected by the low N availability, with a mean reduction of 35%, whereas the dry mass of the roots was differentially affected by the N availability among the studied synthetics, with an increase of the dry mass accumulation under low N availability. Correlation analysis showed that under both N availabilities, there was a strong, positive correlation between uptake efficiency and N use efficiency. Uptake efficiency is an adequate parameter to guide breeding programs seeking N use efficiency in early stages of growth in synthetics maize populations.

Keywords: *Zea mays*, uptake efficiency, utilization efficiency.

1. Introduction

Nitrogen (N) is the most required nutrient in maize crop, and its deficiency is one of the primary reasons for the discrepancy between potential yield and world average yield, which reaches 9,000 kg ha⁻¹ (LOBELL et al., 2009). Low N availability is a severe constraint, especially in developing countries (HU et al., 2008). In developed countries, the intensive use of N fertilizers raises the economic and energy costs of production systems and potentially polluting the environment (MISHIMA et al., 2011).

In this context, the development of cultivars with high N use efficiency (NUE) is desirable, since it would allow the attainment of adequate yields in soils with low N availability. Additionally, the development of such cultivars would enable the reduction of the amount of fertilizer applied in high yield systems, reducing the cost of production and the negative environmental impact of this fertilizer.

NUE was defined by Moll et al. (1982) as the amount of grain produced as a function of the amount of N available. NUE can be deployed in two components: uptake efficiency (NUpE) and utilization efficiency (NUtE), with NUpE being more important under high N availability, and NUtE being more important under low N availability. However, these data were obtained from flowering plants, and the same attributes may not be true for early stages. Uptake efficiency is related to the ability of the genotype in absorbing the N available in the soil, while NUtE is the ability of transforming the N absorbed in a biological product.

Maize yield begins to be defined in the V₄ stage (four leaves fully expanded), when tassel initiation occurs, followed by ear initiation on internodes in V₆ and tassel elongation in V₈ (HANWAY, 1966). According to Hirel et al. (2001), the yield increase in maize genotypes is directly associated with a higher N accumulation in the leaves during the early stages. Therefore, NUE from the early stages is important to ensure high crop yields.

The genetic gain in maize, especially under conditions of low N availability, is slow due to the lack of genetic variability (TALABI et al., 2017). Therefore, it is important to identify sources of variability whose genes related to NUE may be introduced into breeding programs. In this context, it is essential to generate more detailed information on the components of NUE and its relationships with the traits of maize plants in early stages, as well as to evaluate the most suitable environment for selection to guide breeding programs.

The objective of this study was to identify the traits related to NUE in the early stages of synthetic maize populations, based on the components of NUE, dry mass accumulation, and index of chlorophyll content.

2. Material and Methods

The experiment was conducted in Jaboticabal, São Paulo, Brazil (21°14'33"S, 48°17'10"W, at 565 m asl). Two synthetic maize populations and the commercial open-pollinated variety AL Avaré (as control) were used. The synthetics were previously studied in a 2-year field experiment, and the synthetic selected were part of the group with high (A) or low yield (B). The treatments were arranged in a 3 × 2 factorial scheme,

being three maize genotypes and two contrasting levels of N, distributed in a randomized complete block design with five replications, totaling 30 plots. The plots were represented by two pots with four plants each, totaling 8 plants per plot.

2.1. Application of N treatments

The contrasting N levels were simulated by applying 100 and 400 mg dm⁻³ of N in treatments under low and high N availability, respectively (CARVALHO et al., 2012). All plots received 100 mg dm⁻³ of N via urea at sowing, which was incorporated into the pots. In the plots that corresponded to the high N level, the plants received an additional 150 mg dm⁻³ of topdressed N via urea, at stages V₃ and V₅, totaling 300 mg dm⁻³ of topdressed N. Topdressed urea was applied in diluted form to ensure incorporation.

The pots, with a capacity of 12 dm⁻³, were filled with 10 dm⁻³ of air dry soil and sieved using a 5-mm mesh. Soil collection was conducted in subsoil classified as eutroferric red oxisol. Prior to the installation of the experiment, soil chemical analysis was performed, which presented the following characteristics: pH (CaCl₂) = 6.1; organic matter = 14.5 g dm⁻³; P (resin) = 9.5 mg dm⁻³; S = 16.5 mg dm⁻³; H + Al = 15.5 mmol_c dm⁻³; K = 1.0 mmol_c dm⁻³; Ca = 19.0 mmol_c dm⁻³, Mg = 5.5 mmol_c dm⁻³; CTC = 41.3 mmol_c dm⁻³, V = 63%. Soil correction was not necessary.

2.2. Experimental conduction

The experiment was conducted in open air area in March and April 2017. The maximum and minimum temperatures observed during the conduction of the test were 34.3°C and 13.5°C, respectively. Sowing fertilization was done 12 d prior to sowing the genotypes and included (apart from the 100 mg dm⁻³ of N) 450 mg dm⁻³ of P and 150 mg dm⁻³ of K. P was incorporated throughout the pots, while K was incorporated in the surface along with N. During pot preparation and conduction of the experiment, pot moisture was maintained at 80% of the field capacity.

On the first N topdressing, performed in the V₃ stage in plots that corresponded to the high N level, 50 mg dm⁻³ of K was also applied in all experimental plots. Together

with the second topdressing in the V₆ stage, the application of S, Mg, B, Mn, Mb, and Zn was carried out in all plots of the experiment.

2.3. Chlorophyll content index

The chlorophyll content index was estimated using the portable chlorophyll meter CCM-200 (OPTI-SCIENCES) in all plants of each plot. Measurements were taken on the fully expanded eighth leaf during the V₈ stage.

2.4. Plant analysis

The plants were collected in the V₈ stage, separating the shoots and roots, which were washed under running water and then in distilled water. The plant material was dried in a ventilated oven at 75°C for 72 h and weighed to determine the shoot and root dry masses. Afterwards, the samples were ground together (shoot + root) and submitted to laboratory analysis to determine the N content in the whole plant, using the Kjeldahl method. The total N content was obtained by multiplying the N content in the plant by the total dry mass.

The NUE, NUpE, and NUtE were calculated, with $NUE = \text{total dry mass} / N \text{ applied}$; $NUpE = (\text{total N content} \times 100) / N \text{ applied}$; $NUtE = \text{total dry mass} / \text{total N content}$ (Carvalho et al., 2012).

2.5. Statistical analysis

Individual variance analysis was performed to obtain the mean square values of the genotype (MS_{gen}) and mean square of the residue (MS_e), and the values were used to calculate the selective accuracy: $SA = [1 - 1 / (MS_{gen} / MS_e)]^{0.5}$ (RESENDE; DUARTE, 2007) and coefficient of determination: $R^2 = MS_{gen} / (MS_{gen} + MS_e)$.

The means were analyzed by the Student's t-test, comparing the performance of each genotype in the contrasting environments and of all genotypes, in pairs, in each of the environments. The Pearson's correlation analysis between the traits was also

conducted, generating a heat map of the correlations for each N availability. All statistical analysis was performed using R software (R CORE TEAM, 2014).

3. Results

3.1 Precision measures

The selective accuracy ranged from 0.62–0.87 in the environment with high N availability and from 0.81–0.98 in the low availability environment. The coefficient of determination, in turn, was 0.29–0.80 under high N availability and 0.05–0.91 under low availability. For both parameters, the traits that presented the lowest and highest values were, respectively, NUtE and chlorophyll content index under high N and NU_pE and dry mass of the shoot under low N (Table 1).

Table 1. Mean square of genotype (MS_{gen}), mean square of residue (MS_e), selective accuracy (SA), and coefficient of determination (R^2) of chlorophyll content index (CCI), shoot dry mass (SDM), root dry mass (RDM), nitrogen uptake efficiency (NU_pE), nitrogen utilization efficiency (NU_tE) and nitrogen use efficiency (NUE) of two experimental synthetics and a commercial variety of maize, under high (HN) and low (LN) nitrogen availability.

	CCI	SDM	RDM	NU _p E	NU _t E	NUE
HN						
MS_{gen}	10.03	71.30	94.70	19.48	27.59	6.08
MS_e	2.43	31.57	27.05	48.09	17.12	3.00
SA	0.87	0.75	0.85	- ¹	0.62	0.71
R²	0.80	0.69	0.78	0.29	0.62	0.67
LN						
MS_{gen}	0.25	208.93	153.92	482.90	3.21	424.64
MS_e	0.48	21.42	29.42	167.77	60.57	75.68
SA	- ¹	0.98	0.90	0.81	- ¹	0.91
R²	0.34	0.91	0.84	0.74	0.05	0.85

¹ it was not possible to calculate the SA due to the low value of MS_{gen}/MS_e

3.2. Chlorophyll content index

The chlorophyll content index was, on average, 62% higher in the environment with high N availability, considering all genotypes. Under high N availability, synthetic A presented the highest chlorophyll content index, 21.52, while synthetic B and AL Avaré presented values 7% and 13% lower, respectively. Under low N availability, there was no difference between the chlorophyll content index of the genotypes (Table 2).

Table 2. Means of chlorophyll content index (CCI), shoot dry mass (SDM), root dry mass (RDM), nitrogen uptake efficiency (NUpE), nitrogen utilization efficiency (NUtE) and N use efficiency (NUE) of two experimental synthetics and a commercial variety of maize, under high (HN) and low (LN) nitrogen availability.

		CCI ¹	SDM	RDM	NUpE	NUtE	NUE
HN	A	21.52 Aa	53.59 Ab	30.57 Bb	51.28 Ba	41.50 Ba	21.04 Ba
	B	19.95 Ab	61.04 Aa	28.30 Ab	48.92 Ba	46.09 Ba	22.34 Ba
	Avaré	18.70 Ab	56.24 Ab	36.71 Aa	52.84 Aa	44.67 Ba	23.24 Ba
SD		1.76	6.61	5.76	7.41	5.12	2.21
LN	A	7.65 Ba	36.42 Bb	38.39 Aa	79.34 Aa	94.07 Aa	74.81 Aa
	B	7.27 Ba	43.47 Ba	30.27 Ab	79.62 Aa	93.24 Aa	73.74 Aa
	Avaré	7.66 Ba	30.56 Bb	27.78 Bb	62.46 Ab	94.84 Aa	58.34 Ab
SD		0.65	6.85	7.64	14.00	6.39	11.72

¹ Means followed by the same capital letter do not differ between the availabilities of N by the t-test. Means followed by the same lowercase letter do not differ from each other within the same N availability by the t-test.

3.3. Shoot and root dry mass accumulation

Shoot dry mass in all genotypes was affected by low N availability, with a mean reduction of 35%. Synthetics A and the control AL Avaré did not differ for this trait under high N availability. A similar scenario was observed under low N availability, where

synthetic B accumulated 43.47 g in the shoot, and synthetics A and variety Avaré accumulated 23% less (Table 2).

The root dry mass was differentially affected by the N availability among the genotypes studied. Synthetic A showed an increased root dry mass accumulation of 20% under low N availability, while the control Avaré showed a decrease of 24% under the same conditions. Synthetic B showed no change in root dry mass accumulation under low N availability, compared to performance under high N availability.

Analyzing the behavior of the genotypes under high N availability, the control Avaré had a highest accumulation of root dry mass, and the synthetics obtained values an average of 19% lower. Under low N availability, synthetic A accumulated 38.39 g of root dry mass, while synthetics B and control Avaré accumulated 21% and 28% less dry mass, respectively (Table 2).

3.4. NUE, NUpE, and NUtE

On average, NUpE was significantly higher under low N availability for the synthetics, while the control Avaré averaged 57% of the N applied, regardless of the level of nutrient availability (Table 2).

Under high N, the genotypes absorbed 51% of the applied amount of the nutrient, not having significant difference between them. Under low N availability, the AL Avaré had the lowest NUpE, absorbing 62% of the N applied, while synthetics A and B absorbed 79% of the nutrient (Table 2).

The NUtE under high N availability was 44.09 g per g of absorbed N and was 2.1-fold higher for all genotypes in the environment with low N availability. There was no difference between genotypes within the environments (Table 2).

The NUE under high N availability averaged 22.21 g dry mass accumulated per gram of nutrient applied, while under low N availability, the accumulation was 68.96 g, representing a 68% increase of NUE when N was available in a lower quantity. There was no difference between genotypes under high N availability. Synthetics presented superior performance to the control under low N availability, accumulating 21% more total dry mass per unit of applied N when compared to the variety Avaré (Table 2).

Analyzing the mean values of all evaluated traits under high N revealed that the synthetics and the control demonstrated similar behavior. One exception is for root dry mass (Figure 1A), which had a higher accumulation for the control compared to synthetics (Table 2). Under low N availability, the control AL Avaré presented a decreased ability regarding the evaluated traits, and the synthetics stood out for shoot and root dry mass, NUpE, and NUE (Figure 1B).

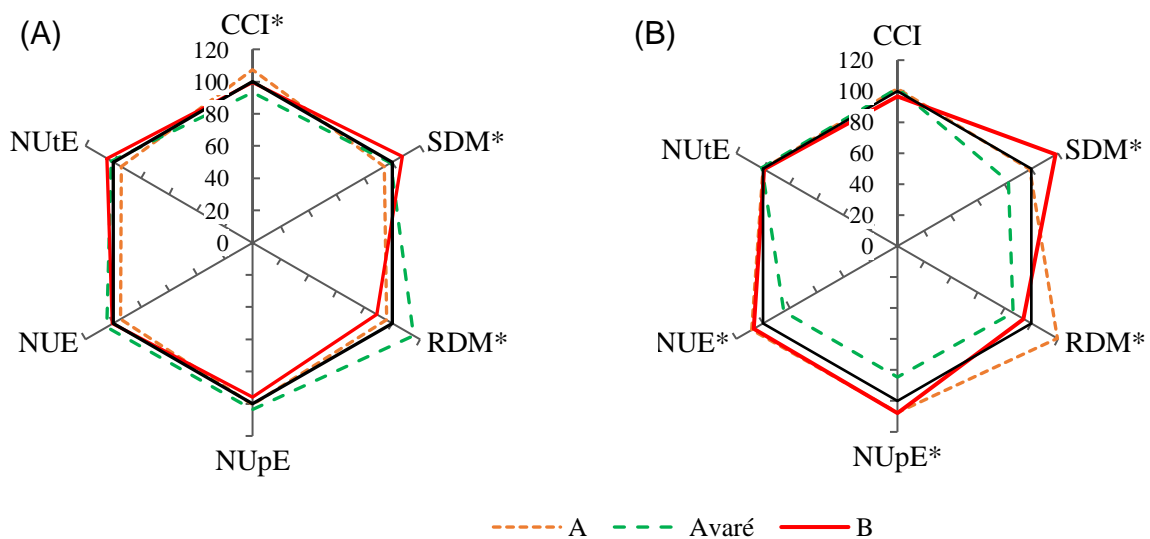


Figure 1. Phenotypic difference between two experimental synthetics and the control AL Avaré under high (A) and low (B) N availability. The difference for each trait is indicated by the relative increase or decrease in percentage, in relation to the average of the three genotypes. Significant differences between at least two genotypes are represented by an asterisk, and the black solid line represents the average of the three genotypes for the trait. Chlorophyll content index (CCI), shoot dry mass (SDM), root dry mass (RDM), nitrogen uptake efficiency (NUpE), nitrogen utilization efficiency (NUtE) and N use efficiency (NUE).

3.5. Correlations

All correlations considered strong were also significant according to the Student's t-test at 5% probability. The correlations between the traits were generally stronger and predominantly positive in the environment under low N availability, while under high availability, they were weak or null and often negative (Figure 2).

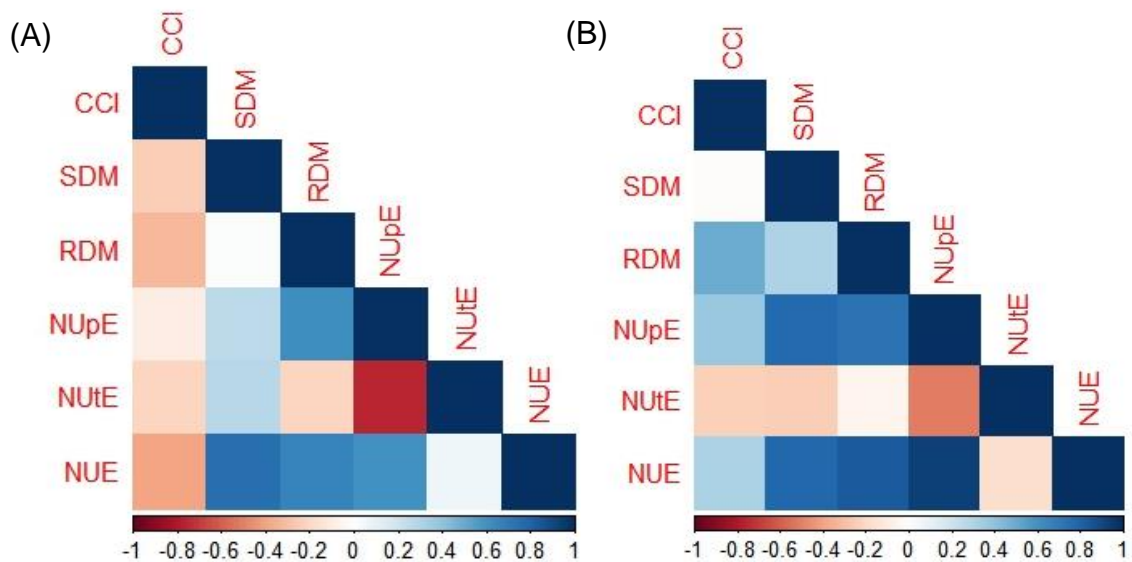


Figure 2. Heat map of correlations between chlorophyll content index (CCI), shoot dry mass (SDM), root dry mass (RDM), nitrogen uptake efficiency (NUpE), nitrogen utilization efficiency (NUtE) and nitrogen use efficiency (NUE), under high (A) and low (B) nitrogen availability in maize genotypes. The darker the color, the greater the correlation, where blue tones represent positive correlations and red tones represent negative correlations.

Under high N availability, there was a strong and positive correlation between root dry matter and NUpE, and shoot/root dry matter and NUpE with NUE, as well as strong and negative correlation of NUtE with NUpE (Figure 2).

Under low N availability, there was a strong and positive correlation between shoot/root dry mass with NUpE, and shoot/root dry mass and NUpE with NUE. There was no correlation between NUtE and NUE (Figure 2).

4. Discussion

The selective accuracy is an adequate parameter to evaluate the experimental accuracy. Its use is recommended over the coefficient of variation, as the selective accuracy is independent of the average of the experiments (SILVA et al., 2013). Thus, under high N availability, the experimental precision was classified as high for the evaluated traits, except for NUtE, which presented moderate precision. Under low N availability, the experimental precision was classified as high or very high according to Cargnelutti Filho et al. (2012).

Regarding the coefficient of determination, values greater than 0.80 indicate that the variability is due to genotype, rather than the environment (CARGNELUTTI FILHO; STORCK 2007), meaning that the probability of selection caused by genotypic effects is high. Under high N availability, the only trait that fulfilled this assumption was the chlorophyll content index. Under low N availability, shoot dry mass, root dry mass, and NUE obtained values above 0.80.

The difference in chlorophyll content index between the environments indicates that the N rates used were sufficient for distinguishing the environments for N availability, but the chlorophyll content index was not adequate for discriminating the genotypes. Similar results were found by Vergara-Díaz et al. (2016), which determined that the chlorophyll content index is one of the best predictors of leaf N content but is not efficient for discriminating genotypes.

Shoot dry mass of all genotypes was negatively affected by the low availability of N. When studying the effect of the lack of N in the proteome of maize, Wei et al. (2015) observed more than 20 proteins that had lower expression, among them ribulose-1,5-bisphosphate and glutamine synthase, which are key enzymes for photosynthesis and N metabolism, respectively. Therefore, a lower contribution of N decreases the shoot dry mass.

The same was not observed for root dry mass, which was differentially affected between genotypes. Similar results were reported by Elazab et al. (2016) in wheat, which observed a decrease in shoot dry mass and an increase in root dry mass under conditions of low N availability. The decrease of the shoot dry mass and increase of the root dry mass, resulting in a lower shoot/root ratio, can be related to an adaptive

response to increase N uptake, while simultaneously retaining the already absorbed N to maintain metabolism, even under stress (SEN et al., 2016). In this way, the synthetics present greater adaptation to environments with low N availability, whereas the control Avaré is more suitable for environments with high N availability.

This response was already expected, as high N doses are used in the experiments to select the most productive maize cultivars (MARTINS et al., 2008); therefore, commercial cultivars have high yields but are generally not efficient regarding N use. Consequently, these genotypes tend to present improved performance in environments with high N availability compared to genotypes that have not yet been selected under these conditions.

NUpE was higher under low N availability for the synthetics, whereas the control did not show a difference in the uptake capacity among N availability. Although root development is often associated with a higher N uptake capacity (CHEN et al., 2013), the accumulation of shoot dry mass can also be a determining factor, since it leads to a higher demand of N (PANG et al., 2015). In *Arabidopsis*, lateral root development and induction of the NRT2.1 gene — a nitrate carrier — are regulated by signals from the shoot, evidencing that the increased N uptake is not related only to the root system (LIU et al., 2015). NUtE was constant among genotypes, but higher in the environment under low N availability. According to Do Vale et al. (2011), one of the explanations for this would be the antagonistic effect between NUpE and NUtE.

The increase of the root uptake capacity is one of the key points to increase NUE, since it is common for N-inorganic added to the system to be superior to the root uptake capacity, which implies losses (BRACKIN et al., 2015). Li et al. (2015) identified over 150 quantitative trait loci (QTLs) that influence NUE and root system architecture, and 70% of these QTLs coincide with these two characteristics. Moreover, the authors observed a positive and significant correlation between the root dry mass at the V₅₋₆ stage and the NUE calculated from the grain yield. It is worth mentioning that root dry mass is not the only character related to the greater capacity of nutrient uptake, but it is the easiest to be measured.

The results obtained in this work indicate that under high N availability, the increase of root dry mass in the early stages would result in an increased NUpE, while under low N availability, both the shoot and the root system positively affect NUpE of

plants. However, in this case, NUtE-focused improvement is not indicated to increase NUE, since these parameters are not correlated at early stages. Thus, the evaluation of NUE can be done by evaluating the root dry mass in the V₈ stage, and the genotypes with the highest dry mass accumulation will be the most efficient in the N use due to the higher NUpE, regardless of the availability of N.

Environments with low N availability are best suited for evaluation and selection for NUE. The genetic control of quantitative traits under low N availability is more complex than under high availability (RIBAULT et al., 2007), and it is very likely that selection under low N conditions will not negatively affect the traits under high N (LAFFITE; EDMEADES, 1994).

5. Conclusions

We conclude that the synthetics present higher NUE when compared to the commercial control. The root dry mass accumulation is an adequate trait to evaluate NUE in the early stages regardless of N availability. Although this parameter is suitable for evaluation in both environments, the low N availability environment is adequate for discriminating the genotypes via root dry mass. Future studies should evaluate the correlation of vegetative efficiency with grain yield to verify the feasibility of using such parameters for early genotype selection.

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CAPÍTULO 4 – Considerações Finais

Os sintéticos estudados apresentam potencial para uso sob baixa disponibilidade de N, pois não apresentam diferenças expressivas entre os caracteres estudados sob alta ou baixa disponibilidade de N. Considerando que não sofreram processo de seleção e que foram semeadas em baixa densidade populacional, alguns sintéticos atingiram níveis de produtividade superiores à média nacional e à testemunha comercial, além de apresentarem baixo percentual de acamamento. Sob condições de restrição do desenvolvimento radicular, nós observamos que a baixa disponibilidade de N afeta o acúmulo de massa seca da parte aérea e principalmente das raízes, e que a eficiência de absorção dos sintéticos é aumentada.

Considerando que não foi observada diferença de produtividade a campo, mas que o acúmulo de massa seca é alterado em função da disponibilidade de N, podemos imaginar que a absorção de N a campo, condição onde o desenvolvimento radicular não é restrito, foi suficiente para assegurar a obtenção dos mesmos níveis de produtividade que foram obtidos sob alto N. Essa informação, associada aos relatos da literatura de que sob baixa disponibilidade de N, as raízes de alguns genótipos podem assumir desenvolvimento mais íngreme para atingir camadas mais profundas, nos leva a imaginar que os sintéticos apresentam alta capacidade adaptativa à ambiente com baixos níveis de N, aumentando a absorção do nutriente. Como próximo passo, seria interessante estudar a morfologia e desenvolvimento do sistema radicular dos sintéticos e, além disso, o proteoma, para verificar se não há alteração da expressão de proteínas entre alta e baixa disponibilidade de N.