DAYANE MEIRELES DA SILVA

CARACTERIZAÇÃO FISIOLÓGICA DA DEFICIÊNCIA DE MAGNÉSIO EM MUDAS DE *Coffea arabica* L. SUBMETIDAS AO CALOR

LAVRAS – MG
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Prof. Dr. José Donizeti Alves
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PHYSIOLOGICAL CARACTERIZATION OF MAGNESIUM DEFICIENCY IN *Coffea arabica* L. SSEEDLINGS SUBMITTED TO HEAT STRESS

Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Agronomia/Fisiologia Vegetal, para a obtenção do título de Doutor

APROVADA em 17 de fevereiro de 2016.

Prof. Dr. José Donizeti Alves – UFLA

Prof. Dr. Luiz Edson Mota de Oliveira – UFLA

Prof. Dr. Paulo Eduardo Ribeiro Marchiori – UFLA

Dra. Milene Alves de Figueiredo Carvalho – EMBRAPA/Café

Dra. Vânia Aparecida Silva – EPAMIG

Prof. Dr. José Donizeti Alves

Orientador

LAVRAS – MG

2017

RESUMO

O objetivo do presente estudo foi investigar a importância da nutrição adequada de magnésio em mudas de *Coffea arabica* L. cv. Catuaí submetidas a elevadas temperaturas. Para tanto, mudas de cafeeiro foram transferidas para recipientes plásticos contendo solução nutritiva com diferentes concentrações de magnésio (com e sem magnésio) e submetidas a duas temperaturas (25 e 35 °C). Folhas totalmente expandidas e raízes foram coletadas no início do período experimental e após 10, 20 e 30 dias. As análises realizadas foram: produção de massa seca, quantificação de magnésio, clorofila total, açúcar solúvel total, açúcar redutor, sacarose, amido, peróxido de hidrogênio, prolina, ascorbato, malondialdeído, proteína e aminoácido, além da atividade das invertases ácida do vacúolo, ácida da parede celular e neutra do citosol; dismutase do superóxido, catalase, peroxidase do ascorbato, redutase do monodehidroascorbato, redutase do dehidroascorbato e redutase da glutationa. As variáveis analisadas foram impactadas principalmente pela combinação dos estresses, deficiência de magnésio e calor. Tanto cada um deles isoladamente, como a combinação deles culminou em um aumento na relação de massa seca entre parte aérea e raiz, o que pode estar relacionada ao acúmulo de carboidratos nas folhas e a sua redução no tecido radicular. A atividade das invertases foi negativamente afetada por ambas os estresses, sendo a combinação deles responsável pelas menores atividades. Com uma menor atividade das invertase, o tecido radicular tem sua atividade reduzida e consequentemente sua força em atrair fotoassimilados fica comprometida. Além disso, a deficiência de magnésio e o calor desencadearam uma maior produção de peróxido de hidrogênio, o que foi acompanhado por uma maior atividade do metabolismo antioxidante e pela maior produção de prolina e ascorbato. No entanto, o metabolismo antioxidante foi ineficiente na remoção do excesso de espécies reativas de oxigênio produzidas, resultando em elevada peroxidação lipídica e degradação proteica. Quando submetidas ao calor, mudas de cafeeiro bem nutridas em magnésio apresentaram menores danos fisiológicos do que aquelas deficientes em magnésio. Dessa forma, atenção deve ser dada em relação à nutrição em magnésio em mudas de *Coffea arabica* L. cv. Catuaí a fim de mitigar as perdas em decorrência da elevação da temperatura.

Palavras chave: Particionamento de carboidratos. Invertases. Metabolismo antioxidante. Prolina.

ABSTRACT

Aimed with this study investigate the importance of adequate magnesium nutrition in *Coffea arabica* L. seedlings cv. Catuaí submitted to heat stress. In this way, seedlings were transferred to plastic recipient contain a nutrient solution with different magnesium concentrations and subjected to two temperatures (25 and 35 °C). Fully expanded leaves and roots were collected at the beginning of the experimental period and after 10, 20 and 30 days. Variable analyzed were dry weight production, magnesium, total chlorophyll, total soluble sugar, reducing sugar, sucrose, starch, hydrogen peroxide, proline, ascorbate, malondialdehyde, protein and amino acid, invertases activity, dismutase of superoxide, catalase, ascorbate peroxidase, monodehydroascorbate reductase, dehydroascorbate reductase and glutathione reductase. These analyzed variables were affected mainly by the combination of stress, Mg-deficiency and heat stress. Alone or in combination these stresses increase shoot:root dry weight ratio, which may be related to carbohydrates accumulation in leaves and its reduction in roots. Invertases activity was negatively affected by both stresses, being the combination of these stresses responsible for the lowest activities. With this decrease in invertase activity, roots showed a reduced activity and consequently its strength in attracting photoassimilates stayed compromised. In addition, Mg deficiency and heat stress caused an increase in hydrogen peroxide concentration, which was accompanied by a higher activity of antioxidant metabolism and higher production of proline and ascorbate. However, the antioxidant metabolism was inefficient in reactive oxygen species removal, resulting in high lipid peroxidation and protein degradation. When submitted to heat, coffee seedlings with Mg showed lower physiological damages than those Mg-deficient. Therefore, greater attention is required about magnesium nutrition in *Coffea arabica* L. seedlings cv. Catuaí in order to mitigate the losses due to heat stress.

Keywords: Carbohydrate partitioning. Invertases. Antioxidant metabolism. Proline.

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PRIMEIRA PARTE

1. INTRODUÇÃO

O estresse por calor tem se tornado uma crescente preocupação, visto o quadro atual de aquecimento global e os prejuízos causados na produtividade de muitas culturas (LUO et al., 2014). A elevação na temperatura desencadeia perturbações em vários processos fisiológicos e metabólicos, como a alteração no particionamento de carboidratos para o tecido radicular e o consequente prejuízo no seu crescimento (DU; TACHIBANA, 1994).

Além dos prejuízos ao desenvolvimento radicular, o desequilíbrio na relação fonte-dreno compromete o processo fotossintético, favorecendo a superprodução de espécies reativas de oxigênio (EROs; ALMESELMANI et al., 2006). O acúmulo de EROs causa peroxidação dos lipídeos de membrana, desnaturação de proteínas e mutações no DNA (MARUTANI et al., 2012). Para proteger as células dos danos oxidativos, os vegetais intensificam seu metabolismo antioxidante, tanto o enzimático como o não enzimático. Fazem parte do primeiro, as enzimas dismutase do superóxido (SOD), catalase (CAT), peroxidase do ascorbato (APX), redutase do dehidroascorbato (DHAR), redutase do monodehidroascorbato (MDHAR) e redutase da glutationa (GR). Enquanto que o segundo é composto por moléculas com função antioxidante, como ascorbato e a glutationa (GILL; TUTEJA, 2010).

O aumento na produção de espécies reativas de oxigênio, bem como a interrupção da translocação de fotoassimilados de órgãos fonte para órgãos dreno são também muito comuns em plantas sob deficiência mineral, especialmente a de magnésio (Mg; CAKMAK, 2013; MENGUTAY et al., 2013; WARAICH et al., 2012; SILVA et al., 2014). Foi anteriormente relatado que em resposta à deficiência de Mg, mudas de *Coffea arabica* L. da cultivar Catuaí apresentaram elevação na concentração de fotoassimilados no tecido foliar e redução de seus níveis no tecido radicular, além de alterações no metabolismo antioxidante em decorrência da elevação na produção de EROs e a consequente redução no crescimento vegetal, com destaque para o sistema radicular (SILVA et al., 2014).

Como a elevação na temperatura é uma realidade dos dias atuais, caso não seja dada a atenção necessária à nutrição vegetal, inclusive no que diz respeito à nutrição por

magnésio, grandes poderão ser as perdas na produtividade do cafeeiro quando for submetido a combinação desses estresses. Por outro lado, já existem muitos relatos de cafeicultores que apontam sérias reduções na produção de café associadas à deficiência de Mg. Esses relatos são comuns no Sudoeste da Bahia, Norte de Minas Gerias, Triângulo Mineiro e em regiões de baixa altitudes no Sul de Minas Gerais, que são áreas onde notadamente predominam temperaturas elevadas. Pelo exposto, parece que a interação dos estresses, deficiência de Mg e altas temperaturas, pode ser considerada como uma das principais causas do baixo crescimento e desenvolvimento dos cafeeiros naquelas regiões.

Dessa forma, o objetivo do presente estudo foi investigar a importância da nutrição adequada de magnésio em mudas de *Coffea arabica* L. cv. Catuaí submetidas a elevadas temperaturas.

2. REFERENCIAL TEÓRICO

2.1 Nutrição por magnésio no cafeeiro

O magnésio (Mg) é um macronutriente que desempenha várias funções nos vegetais. Dentre as principais funções está sua posição central na molécula de clorofila, sua participação na síntese de proteínas e na ativação de inúmeras enzimas envolvidas na respiração, fotossíntese e, principalmente, aquelas envolvidas na transferência de grupos fosfatos (fosfatases e ATPases) (MARSCHNER, 2012). Logo, a deficiência de Mg é capaz de prejudicar uma gama de processos fisiológicos e assim afetar o crescimento e o desenvolvimento do vegetal.

Amostras de solos e de folhas oriundas de diversas regiões cafeeiras, encaminhadas para a Fundação Pro Café de Varginha – Minas Gerias, indicaram que o Mg é o nutriente em maior déficit nas lavouras cafeeiras (DIAS, 2015). Inclusive, a baixa produtividade de alguns talhões de lavouras cafeeiras do Oeste baiano já foi correlacionada com os baixos níveis de Mg encontrados no solo desses talhões (ALMEIDA; LIMA; AMARAL, 2006).

O desequilíbrio na relação entre cálcio (Ca), potássio (K) e magnésio é apontado como uma das principais causas da deficiência de Mg em lavouras cafeeiras. Isso acontece porque tais nutrientes competem por sítios de absorção nas raízes, e dessa forma o excesso de um pode inibir a absorção do outro (MALAVOLTA, 1996). O desequilíbrio

na relação entre Mg e K tem sido também associado ao uso constante de doses elevadas de adubos ricos em K e a não aplicação de Mg. Em lavouras cafeeiras com baixa produtividade foi observada, por meio de análises da fertilidade do solo, a combinação da deficiência de Mg e do excesso de K (ALMEIDA, LIMA, AMARAL, 2006).

Além disso, grande parte das lavouras cafeeiras do Brasil estão implantadas em solos ácidos, com baixa disponibilidade de Ca e Mg, e que portanto necessitam de calagem. No entanto, em algumas lavouras tal prática não é adotada, ao passo que em outras onde a calagem constitui uma prática de uso generalizado, ainda persistem dúvidas quanto à dosagem, ao efeito residual e à localização ideal da aplicação. O calcário vem sendo utilizado como o principal agente corretivo de pH e a fonte mais usual e econômica de fornecimento de Ca e Mg. No entanto, muitos calcários apresentam baixos teores de Mg, além de baixa reatividade e solubilidade, o que limita o fornecimento do nutriente para o vegetal (TOMAZ et al., 2003).

Acredita-se que a baixa produtividade de lavouras cafeeiras implantadas em solos de Cerrado, caracterizados como de baixa fertilidade natural, está principalmente associada ao manejo inadequado da fertilidade do solo (MATIELLO et al., 2010). Mesmo diante disso, poucos ainda são os trabalhos que buscam elucidar os impactos da deficiência de nutrientes, especialmente do Mg, em uma cultura tão economicamente importante como a do café.

2.2 Deficiência de Mg e o particionamento de carboidratos

Um dos principais efeitos da deficiência de Mg na maioria das espécies é a alteração no particionamento de carboidratos e na alocação de biomassa entre parte aérea e raiz. Dentre as características iniciais da deficiência de Mg está o aumento na relação de massa seca entre parte aérea e raiz, o que está relacionado ao baixo fornecimento de carboidratos para o tecido radicular (CAKMAK; KIRKBY, 2008; KOBAYASHI et al., 2012; VERBRUGGEN; HERMANS, 2013; SILVA et al., 2014). Em cafeeiros da cultivar Catuaí já foi relatada a elevação na concentração foliar de fotoassimilados, bem como a redução de seus níveis no tecido radicular, em resposta à deficiência de Mg; o que desencadeou uma elevação na relação de massa seca entre parte aérea e raiz (SILVA et al., 2014).

No entanto, as causas dessa redução na translocação de sacarose em plantas deficientes em Mg ainda permanecem obscuras. Acredita-se que os órgãos dreno podem ter sua atividade reduzida, exercendo menor força na translocação de sacarose em sua direção (FISCHER et al., 1998). Ou então, que o problema pode estar no processo de carregamento de sacarose no floema (CAKMAK; KIRKBY, 2008; GRANSEE; FHÜRS, 2013; MENGUTAY et al., 2013).

Envolvidas no processo de particionamento de carboidratos estão as enzimas sintase da sacarose fosfato (SPS), as invertases e a sintase da sacarose (Susy). A SPS é uma enzima chave na biossíntese de sacarose em tecidos fotossintéticos e não fotossintéticos. Por outro lado, as invertases e a Susy catalisam a hidrólise da sacarose em hexoses disponibilizando carbono e energia para a manutenção celular (KOCH, 2004).

O transporte de fotoassimilados de órgãos fonte para órgãos dreno é impulsionado por diferenças nos potenciais ao longo do floema. Isso ocorre porque o carregamento de sacarose no floema aumenta a concentração de sacarose no floema causando uma redução no seu potencial osmótico. Essa redução no potencial osmótico do floema em relação ao do xilema faz com que água saia do xilema e vá para o floema desencadeando uma elevação no potencial de pressão (KOCH, 2004). Por outro lado, no dreno o descarregamento de sacarose causa elevação do potencial osmótico do floema e água é perdida para o xilema acarretando em um baixo potencial de pressão. Dessa forma, a sacarose tende a ser transportada por fluxo de massa de um local com alto potencial de pressão para um de baixo potencial de pressão, ou seja, da fonte para o dreno. Assim, a clivagem de sacarose pelas invertases no local de descarregamento do floema mantém o potencial osmótico dessa região alto garantindo o gradiente de potencial que impulsiona o transporte de sacarose em sua direção (KOCH, 2004).

Quando a atividade dessas enzimas, em órgãos dreno, é perturbada a sacarose passa a ser acumulada, reduzindo o potencial osmótico da região e consequentemente elevando o potencial de pressão. Essa mudança no potencial da região do floema próxima ao dreno culmina em uma redução no gradiente de potencial ao longo do floema e consequentemente o transporte de sacarose para esse local fica comprometido. Assim sendo, a deficiência de Mg pode estar relacionada a uma menor atividade dessas enzimas no tecido radicular, o que acarretaria em um menor transporte de sacarose em sua direção.

Por outro lado, o comprometimento na translocação de sacarose pode estar associado efetivamente ao carregamento de fotoassimilados no floema. O processo de

carregamento de sacarose é um processo ativo catalisado pelo cotransporte de H^+ e sacarose, que necessita de um gradiente de prótons através da membrana plasmática das células do floema. O gradiente de prótons necessário para o cotransporte de H^+ e sacarose é estabelecido por uma H^+-ATPase, localizada na membrana plasmática das células de tubo crivado (WARD et al., 1998). O bom funcionamento da H^+-ATPase está relacionado ao Mg-ATP, que é o principal complexo de ATP em sistemas biológicos (IGAMBERDIEV; KLECZKOWSKI, 2003) Logo, uma queda na concentração de Mg-ATP nos locais de carregamento de sacarose no floema pode ser a razão da inibição do transporte de sacarose em folhas deficientes em Mg (CAKMAK; KIRBY, 2008).

2.3 Deficiência de Mg, a produção de EROs e o metabolismo antioxidante

A queda nos teores de clorofilas e o acúmulo de fotoassimilados em folhas de plantas deficientes em Mg pode, mediante mecanismo de *feedback*, inibir o metabolismo fotossintético. Com isso, há uma redução do uso da energia luminosa absorvida que, por sua vez, pode induzir uma saturação da cadeia de transporte de elétrons com acúmulo de NADPH (VERBRUGGEN; HERMANS, 2013; MENGUTAY et al., 2013). Altos níveis de equivalentes redutores e componentes da cadeia de transporte de elétrons saturados oferecem condições favoráveis para a formação de espécies reativas de oxigênio (EROs; MITTLER, 2002). Dentre as principais EROs destacam-se o radical superóxido ($O_2^{\cdot-}$), o peróxido de hidrogênio (H_2O_2), o oxigênio singleto ($^1O_2$) e o radical hidroxila (OH^-) (GILL; TUTEJA, 2010).

As EROs podem reagir com lipídios, proteínas e ácidos nucleicos, causando peroxidação lipídica, desnaturação de proteínas e mutações no DNA, respectivamente (GILL; TUTEJA, 2010). Evidências sugerem que as membranas são o principal alvo da injúria, uma vez que as EROs podem reagir com ácidos graxos insaturados desencadeando a peroxidação lipídica nas membranas plasmática e intracelular de organelas (SHARMA et al., 2012). A peroxidação da membrana plasmática leva ao extravasamento do conteúdo celular, à rápida dessecação e à morte celular, enquanto que danos na membrana intracelular podem afetar a atividade das cadeias de transporte de elétrons da fotossíntese e da respiração (GILL; TUTEJA, 2010).

Assim sendo, as plantas precisam ativar seus sistemas de defesa antioxidante e assim remover as EROs e minimizar seus danos oxidativos celulares. No sistema

antioxidante enzimático, a enzima dismutase do superóxido (SOD) catalisa a dismutação de $O_2^{\cdot -}$ a H_2O_2 e O_2. O H_2O_2, por sua vez, é convertido em O_2 e H_2O pela ação das catalases (CAT), diversas peroxidases e pelo ciclo ascorbato-glutationa. Esse ciclo é caracterizado por uma série de reações redox acopladas, envolvendo as enzimas peroxidase do ascorbato (APX), redutase do monodehidroascorbato (MDHAR), redutase do dehidroascorbato (DHAR), redutase da glutationa (GR) e o ascorbato (GILL; TUTEJA, 2010).

Dessa forma, várias espécies vegetais quando submetidas à deficiência de Mg aumentam seus níveis de moléculas antioxidantes, bem como a atividade de suas enzimas antioxidantes, principalmente SOD, CAT e APX, visando atenuar os danos oxidativos causados pelas EROs (TEWARI et al., 2006; YANG et al., 2012). Especificamente, em *Coffea arabica* L. cv. Catuaí, foi observado um incremento na atividade das enzimas SOD, CAT e APX em resposta à deficiência de Mg (SILVA et al., 2014).

Em condições normais, as EROs são produtos inevitáveis do metabolismo vegetal, e a sua produção e remoção são bem equilibradas. No entanto, em condições de estresse, a produção de EROs pode superar os mecanismos de remoção desencadeando o estresse oxidativo (GILL; TUTEJA, 2010). Em plantas deficientes em Mg, o metabolismo antioxidante muitas vezes não é capaz de remover toda a produção de EROs e vários danos oxidativos podem ser observados. O principal dano das EROs às célula é a peroxidação lipídica, muitas vezes caracterizada pela elevação na produção de malondialdeído, um composto proveniente da peroxidação dos lipídeos de membrana (CAKMAK, 2013; MENGUTAY et al., 2013; WARAICH et al., 2012; SILVA et al., 2014).

2.4 Elevadas temperaturas e o cafeeiro

Estresses abióticos, incluindo alta intensidade luminosa, temperaturas extremas, seca, salinidade e deficiências de nutrientes minerais, são os principais fatores ambientais que resultam em perdas significativas na produtividade de muitas culturas (MENGUTAY et al., 2013). Neste contexto, o estresse por calor é de particular preocupação visto o atual quadro de aquecimento global, em que se é esperado uma elevação de 1 a 6 °C na temperatura média global no século 21 (DE COSTA, 2011). Além disso, períodos de

temperaturas extremamente elevadas devem se tornar cada vez mais frequentes e com duração mais prolongada (MENGUTAY et al., 2013).

A espécie *Coffea arabica* L. é nativa de florestas tropicais da Etiópia em altitudes de 1600 a 1800 m. Nessa região, a temperatura do ar mostra pequenas flutuações sazonais, com média anual de 20 °C (DAMATTA; RAMALHO, 2006). Logo, a temperatura ótima para o seu crescimento varia de 18 a 25 ºC (DAMATTA et al., 2007). No entanto, a temperatura do ar e do solo frequentemente excedem esse nível ótimo, causando injúrias a vários processos fisiológicos e metabólicos. Estudos que abordam o aquecimento global preveem um efeito dramático na cultura do cafeeiro, uma vez que foram estimadas grandes perdas no seu rendimento (GAY et al., 2006), bem como redução da variabilidade genética devido à extinção de linhagens selvagens de *Coffea arabica* L. (DAVIS et al., 2012).

A exposição contínua de plantas de café a temperaturas superiores a 30 °C pode resultar não somente em redução no seu crescimento, mas também no amarelecimento de suas folhas e no crescimento de tumores na base do seu caule (FRANCO, 1958). Temperaturas relativamente altas durante o período de florescimento, especialmente se associado com uma estação de seca prolongada, pode causar o abortamento de flores (CAMARGO, 1985). Dessa forma, através da seleção de cultivares tem sido possível instalar lavouras cafeeiras em regiões com temperaturas médias tão altas quanto 24-25 °C, com rendimento satisfatório (DAMATTA, 2004).

2.5 Elevadas temperaturas e o processo fotossintético

O declínio na concentração de clorofilas em resposta à elevação na temperatura pode ser desencadeado por alterações na estrutura dos tilacóides o que comprometeria a biossíntese de clorofila (DUTTA; MOHANTY; TRIPATHY, 2009), e/ou pela aceleração da sua degradação (MATHUR; AGRAWAL; JAJOO, 2014). Além disso, a redução na concentração das clorofilas pode estar relacionada com a ação oxidativa das espécies reativas de oxigênio sobre as clorofilas (GUO; ZHOU; ZHANG, 2006).

Além da alteração no conteúdo de pigmentos fotossintetizantes, a elevação na temperatura pode afetar o processo fotossintético de outras maneiras. A cadeia de transporte de elétrons e o processo de fixação do CO_2 estão entre os processos mais sensíveis ao calor em vegetais (BERRY; BJÖRKMAN, 1980). O fotossistema II,

juntamente com o seu complexo de evolução do oxigênio, são os principais alvos do estresse por calor (ALLAKHVERDIEV et al., 2008; MARUTANI et al., 2012). Além disso, reduções substanciais na atividade da Rubisco e na performance fotossintética foram observadas em plantas expostas ao estresse por calor (CARMO-SILVA et al., 2012).

2.6 Elevadas temperaturas e o particionamento de carboidratos

A elevação na temperatura tem como principal consequência a redução na translocação de carboidratos e a consequente redução de suas concentração no tecido radicular (TIMLIN et al., 2006; HUANG; RACHMILEVITCH; XU, 2012). Esse tem sido sugerido como o principal fator responsável pela inibição do crescimento radicular e pela interrupção das funções radiculares (DU; TACHIBANA, 1994). Maior suscetibilidade no crescimento das raízes do que da parte aérea, em resposta à elevação da temperatura, foi observado em *Agrostis palustris* (HUANG; GAO, 2000), *Picea mariana* (WAY; SAGE, 2008) e *Triticum aestivum* e *Zea mays* (MENGUTAY et al., 2013).

A fim de sobreviver por longos períodos em altas temperaturas, as raízes devem possuir mecanismos que aumentem a sua eficiência na utilização do carbono quando seu suprimento é limitado. Xu e Huang (2000a, 2000b) observaram que sob alta temperatura o conteúdo de carboidratos não estruturais em raízes de plantas de *Agrostis palustris* tolerantes ao calor foi significativamente maior do que nas plantas sensíveis ao calor. Tal informação sugere que plantas tolerantes ao calor são capazes de manter o fornecimento de carboidratos para o tecido radicular conseguindo assim sustentar o seu metabolismo e crescimento. Li e et al. (2012) sugeriram que a elevação na força do dreno, desencadeada pela maior atividade das invertases, contribuiu para o aumento na tolerância ao calor por essa espécie.

2.7 Elevadas temperaturas, a produção de EROs e o metabolismo antioxidante

Perturbações marcantes na atividade dos fotossistemas e enzimas fotossintéticas de plantas sob estresse por calor limitam severamente a utilização da energia luminosa absorvida. Em tal condição, os cloroplastos são expostos ao excesso de energia de excitação desencadeando uma elevada produção de espécies reativas de oxigênio

(MARUTANI et al., 2012). A superprodução de EROs em decorrência da elevação da temperatura foram observadas em plantas de *Freesia hybrida* (YUAN et al., 2011) e *Triticum aestivum* (KUMAR et al., 2012).

Assim, devido à elevada produção de EROs, o metabolismo antioxidante é ativado visando atenuar os danos oxidativos causados por essas moléculas altamente reativas. A elevação na atividade das enzimas antioxidantes foi observada em plantas de *Cucumis melo* (ZHANG et al., 2014) e *Cucumis sativus* (HUANG et al., 2015) em resposta à elevação da temperatura. No entanto, muitas vezes essa ativação não é suficiente para remover a crescente produção de espécies reativas de oxigênio, e com isso são observados maiores níveis de malondialdeído (MDA), um produto secundário da peroxidação lipídica. Assim sendo, plantas de *Freesia hybrida* (YUAN et al., 2011), *Cucumis melo* (ZHANG et al., 2014) e *Cucumis sativus* (HUANG et al., 2015) quando submetidas à elevação na temperatura apresentaram incrementos na produção de MDA, mostrando que o metabolismo antioxidante não foi capaz de remover a elevada produção de EROs.

2.8 Elevadas temperaturas e a produção de osmoprotetores

Além do metabolismo antioxidante, para conter os efeitos do calor no metabolismo celular, plantas respondem sintetizando e acumulando osmoprotetores. Tais moléculas são pequenas, eletricamente neutras e estabilizam proteínas e membranas contra os efeitos desnaturantes do calor (RIVERO et al., 2014).

Plantas submetidas à alta temperatura usualmente acumulam três tipos de osmoprotetores: betaínas, açúcares não estruturais e prolina (DELAUNEY; VERMA et al., 1993). A prolina também é acumulada em grande quantidade em resposta a outros estresses ambientais (KAVIKISHORE et al., 2005) e parece ter diversos papéis, dentre eles, a estabilização de proteínas, membranas e estruturas subcelulares, bem como na remoção de espécies reativas de oxigênio (KUMAR et al., 2012). Em resposta ao estresse por calor foram observados maiores níveis de prolina em plantas de *Lonicera japonica* (LI et al., 2011) e *Cucumis sativus* (DAI et al., 2012).

3. CONSIDERAÇÕES FINAIS

A interação dos estresses, deficiência de Mg e altas temperaturas, pode estar associada ao baixo crescimento e desenvolvimento dos cafeeiros nas regiões quentes do Brasil. Os principais motivos desse baixo rendimento podem estar relacionados a problemas no particionamento de carboidratos e à elevada produção de EROs, que são alterações primárias no metabolismo de plantas submetidas a esses estresses.

Como a elevação na temperatura é uma realidade dos dias atuais, caso não seja dada a atenção necessária à nutrição vegetal, inclusive no que diz respeito à nutrição por magnésio, grandes poderão ser as perdas na produtividade do cafeeiro quando este for submetido a combinação desses estresses.

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SEGUNDA PARTE

Artigo 1 – Carbon metabolism in *Coffea arabica* L. seedlings submitted to Mg-deficiency and heat stress

Artigo apresentado nas normas da revista Trees (Versão submetida em 17 de janeiro de 2017)

Abstract

Aims Evaluate the impacts of magnesium-deficiency and heat stress on dry weight accumulation, magnesium concentration, chlorophyll levels, sugars concentration and invertases activity in seedlings of *Coffea arabica* L..

Methods Six month old seedlings from *Coffea arabica* L. cv. Catuaí were transferred to plastic containers containing a nutrient solutions with different concentrations of magnesium (with and without) and different temperatures (25 and 35 °C) . Fully expanded leaves and roots were evaluated at the beginning of treatment and after 10, 20 and 30 days for dry weight accumulation, magnesium concentration, chlorophyll levels, sugar concentration and invertases activity.

Results Variables analyzed were mainly affected by the combination of stresses. Mg-deficiency and heat stress was characterized by an increase in shoot/root dry weight ratio, which may be related to accumulation of carbohydrates in leaves and reduction in roots. Invertases activity was negatively affected by both stresses, being the combination of stresses responsible for the lowest values.

Conclusions Greater attention is required in relation to magnesium nutrition in *Coffea arabica* L. seedlings cv. Catuaí in order to mitigate the losses due to heat stress.

Keywords

chlorophyll, sugars, invertases, dry weight

Introduction

Coffea arabica L. originates from sub-forest conditions in Ethiopia, where predominate mild temperatures, low luminosity and good water supply (Guerreiro Filho

et al. 2008). Under sub-forest conditions, coffee plants vegetate more than reproduce, producing small number of grains (Gomes et al. 2008). Brazilian coffee cultivars descend from Ethiopia and, in most cases, are cultivated in full sun. Under this condition, coffee plants are subjected to high temperature and luminosity, as well as low relative humidity. Although, the production of *Coffea arabica* L. fruits, under non-shaded conditions, is relatively high, their growth and development is affected, since most cultivars still preserve physiological attributes of shaded plants. Despite coffee plants have moderate tolerance to drought and full sun (DaMatta et al. 2007; Ronquim 2007), this condition is decisive for accentuating the biennial production and decrease the life cycle of coffee plants (Vaast et al. 2005).

Brazilian coffee crops are frequently submitted to prolonged exposure to irradiances of up to five times its saturation point, high temperatures, dry weather, and low rainfall. This condition can trigger photooxidative damages, commonly known as scalding, characterized by chlorosis and burning of leaves (Oliveira et al. 2012). In coffee plants deficient in magnesium (Mg), leaves exposed to high light radiation intensity showed typical symptoms of scalding (Dias 2015).

Moreover, Mg deficiency in *Coffea arabica* L. plants trigger accumulation of photoassimilates in leaves and reduction in roots, due to interruption of photoassimilates translocation to roots (Silva et al. 2014). The lower supply of photoassimilates to roots leads to increased shoot:root dry weight ratio, restricting the absorption of water and nutrients by roots. As in a vicious cycle, the lower absorption of water and nutrients accentuates chlorosis and leaf burning, with serious damage to productivity.

Recently it was demonstrated that coffee plants under high levels of irradiance have higher physiological requirement of magnesium (Dias 2015). Once there is a direct relationship between irradiance increase and temperature rise, this Mg dependence can also be higher in *Coffea arabica* L. plants exposed to temperature rise. If the rise of 1 to 6 ° C in the average global temperature for the next decades is confirmed (De Costa 2011), more attention should be given to the nutritional status of plants, especially regarding Mg nutrition, considering the greater Mg requirement in such conditions (Dias 2015). In the case of the coffee plants, it is necessary to consider that this culture has been implemented at agricultural frontiers, where the soils are acid, with low cation exchange capacity and therefore poor in magnesium (Tomaz et al. 2003). In addition, Mg deficiency has been observed in plants grown in soils with an imbalance in calcium, magnesium and

potassium ratio, as well as those where potassium fertilization is exceeded (Correa et al. 2001).

In view of temperature rise, if necessary attention is not given to plant nutrition, especially with regard to magnesium nutrition, large losses may occur in coffee productivity when these plants are submitted to a combination of these stresses, heat stress and Mg deficiency. Thus, aimed with this study investigate the importance of magnesium nutrition in *Coffea arabica* L. seedlings cv. Catuaí when submitted to high temperatures.

Material and methods

Plant culture and Mg treatments

Coffea arabica L. seedlings from Catuaí 144 cultivar were cultivated for six months and in polypropylene bags of 500 mL filled with subsoil and cattle manure in 2:1 proportion, plus potassium chloride and superphosphate in the proportion 1:10 (Guimarães et al. 2002). After selection for uniformity in size and vigor, seedlings with four pairs of leaves were transferred to 40 L plastic containers (19x43x66 cm - HxWxL) containing a nutrient solution (Hoaglang and Arnon 1950). Each recipient contained 16 plants. The experiment was conducted in a growth room, with photosynthetic photon flux density around 250 μmol m^{-2} s^{-1} and relative humidity of 50% and 60% during light and dark periods, respectively.

Plants were acclimated for 6 days when solutions with increasing concentrations were used: ¼ strength for 2 days, ½ strength for 2 days, and full strength for 2 days. After six days of acclimation, plants were submitted to four treatments: a) complete nutrient solution with the original concentration of Mg and air temperature of 25 °C (+Mg 25 °C); B) nutrient solution with exclusion of Mg and air temperature of 25 °C (-Mg 25 °C); c) complete nutrient solution with the original concentration of Mg and air temperature of 35 °C (+Mg 35 °C) and d) nutrient solution with exclusion of Mg and air temperature of 35 °C (-Mg 35 °C).

The volume of the nutrient solution was daily replenished with deionized water. The pH of the solution was also adjusted daily to 5.5 ± 0.5 with NaOH and HCL solution (1 mol L^{-1}). The solutions were replaced weekly. All seedlings were maintained under constant aeration throughout the experimental period. The temperature in the root

environment during the completely experimental period was 3 °C lower than that of the leaf environment; and the night temperature was 5 °C below the diurnal temperature, both in the leaf environment and in the root environment.

The design was completely randomized, in a 2x2 factorial scheme, with two treatments (with and without Mg) and two temperatures (25 °C and 35 °C), with five replications. At 0, 10, 20 and 30 days after the imposition of the treatments, completely expanded leaves of the third and fourth pairs and the root system of five plants were collected. Then, plant material was conditioned in liquid nitrogen and stored at -80 °C for analyzes on fresh weight, or brought to the oven at 70 °C under forced circulation to constant weight for analyzes on dry weight.

Variables were analyzed for normality by the Shapiro Wilk Test ($p \geq 0.05$). The variables that fit within the normal range were submitted to analysis of variance, using the SISVAR 4.3 statistical system (Variance Analysis System for Balanced Data) (Ferreira 2011), where the means between the treatments were compared by the Scott test -Knott at 0.05 probability. The variables that did not fit into the normality were transformed by Box-Cox Method and subjected to analysis of variance and mean test by Scott-Knott at 0.05 probability. The non-parametric variables were analyzed by the Kruskal-Wallis Method at 0.05 probability. Pearson correlations were performed between the variables using the statistical program Action Stat (EQUIPE ESTATCAMP 2014).

Shoot and root dry weight

Seedlings were divided into roots and shoots. The plant material was dried at 70°C to constant weight and the dry weight measured.

Evaluation of Mg, chlorophyll and carbohydrates

Magnesium concentration was determined according Malavolta et al. (1989): 500 mg of dry weight were ground and placed in digestion tubes, to which were were added 6 mL of a mix of HNO_3 and $HClO_4$ 2:1 (v/v). The digestion tubes were then put in a block digester and temperature was increased gradually until 160 °C and kept at this temperature until volume of the solution was reduced to half. Temperature was then increased to 210 °C and kept at this temperature until white fumes of $HClO_4$ were

obtained and the extract became colorless. After cooling, the final volume was made up to 50 mL through the addition of deionized water. Determination of magnesium concentration was through atomic absorption spectrometry.

Leaf chlorophyll were determined as described by Lichtenthaler and Buschmann (2001): 0.1 g of fresh weight of the first pair of fully expanded leaves from each treatment were macerated in 80% acetone. The final volume was made up to 10 mL, and spectrophotometric readings were taken at 445, 645 and 663 nm.

Carbohydrates extraction was performed according to Zanandrea et al. (2010): 200 mg of dry weight were homogenized in 5 mL of potassium phosphate buffer 100 mM (pH 7,0) and then placed in a water bath for 30 minutes at 40 °C. Homogenate was centrifuged at 5,000 g for 10 minutes and the supernatant was collected. The process was repeated twice and supernatants were combined. For extraction of starch, the pellet was resuspended in 8 mL of potassium acetate buffer 200 mM (pH 4.8). Then, 16 units of amyloglucosidase enzyme were added and incubated in a water bath at 40 °C for two hours. Following centrifugation at 5,000 g for 20 minutes, supernatant was collected and volume was made up to 15 mL with water. Starch, sucrose and total soluble sugars were quantified as described by Dische (1962), and level of reducing sugars was quantified according to Miller (1959).

Evaluation of invertases activity

Extraction and incubation of soluble invertases (INC: cytosol-neutral invertase and IAV: vacuole acid invertase) were performed as described by Zeng et al. (1999); and insoluble invertase (IAPC: acid invertase of the cell wall) according to Cazetta; Seebauer; Below (1999), with some modifications. INC and IAV extraction was performed by homogenizing 0.4 g of fresh weight in two mL of the extraction buffer containing 200 mM HEPES (pH 7.5), 1 mM PMSF, 5 mM $MgCl_2$, 1 mM DTT and 50 mM ascorbic acid followed by centrifugation at 18,000 g for 20 minutes at 4 °C. The supernatant was collected for analysis of soluble invertases. The pellet was resuspended and homogenized in two ml of sodium citrate buffer 200 mM (pH 4.5), 1 mM PMSF, 25 mM $MgCl_2$, 1 mM DTT, 50 mM ascorbic acid and 1 M NaCl for extraction of IAPC, followed by centrifugation at 18,000 g for 20 minutes at 4 °C, and the supernatant was collected for enzyme analysis.

Enzyme activity was performed in potassium phosphate buffer 100 mM (pH 7.5) for INC and sodium citrate buffer 200 mM (pH 4.5) for IAV and IAPC, plus 5 mM $MgCl_2$ and 200 mM sucrose. The incubation was carried out in a water bath at 30 °C for 40 minutes. The reaction was stopped on ice. Reducing sugars was quantified by DNS method (Miller, 1959) and enzymatic activity was calculated from the difference in reducing sugar concentration at 10 minutes and 40 minutes.

Results

Mg deficiency and the temperature rise reduced dry weight accumulation in shoot and roots (Figure 1). On the 30th day, the exclusion of Mg, under normal temperature (25 ºC), promoted a reduction in shoots dry weigh of 8%, while in roots this decrease was of 23%. Temperature rise, in plants with Mg, triggered a reduction in shoot dry weight of 22% and in root dry weight of 32%. The combination of both stress, Mg-deficiency and heat, leads to a decrease of 38 and 55% on shoot and root dry weight, respectively.

On the other hand, shoot/root dry weight ratio revealed that Mg deficiency was more effective in increasing this ratio than heat stress (Figure 1). In this case, the percentage of increase in the ratio for Mg deficient plants was 20%, while the effect of raising the temperature was 14%.

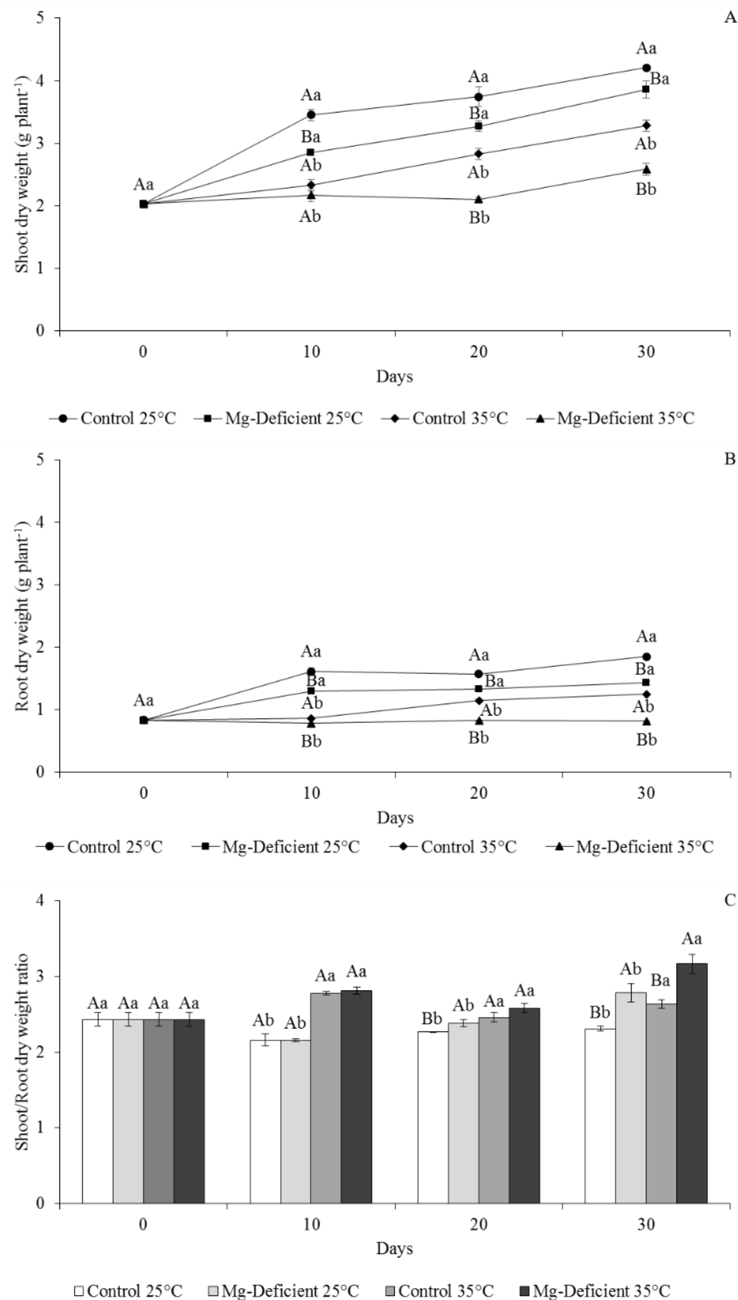


Figure 1: Growth parameters of *Coffea arabica* L. seedlings submitted to different concentrations of magnesium and temperatures. Capital letters compare the effect of magnesium deficiency on each temperature and sampling time and lowercase letters compare the effect of temperature elevation on each Mg condition and sampling time. Different letters indicate significant differences with 0.05 probability. Bars show the standard error of five replicates.

At the beginning of the experiment, magnesium concentration in coffee seedlings cultivated in the presence of this element were 3.48 mg and 3.7 mg Kg^{-1} in leaves and

roots, respectively (Table 1). These values are within the concentration range considered optimum for the coffee plants, ranging from 3.1 to 5.2 mg Kg^{-1} dry weight (Martinez et al. 2003, 2004). In fact, the leaves did not exhibit the typical symptoms of deficiency or excess of Mg. With the evolution of the experiment, Mg concentrations in leaves of seedlings cultivated in the presence of Mg at 25 ºC were practically the same and they did not show symptoms of deficiency (Table 1). On the other hand, when cultivated at 35 ºC (+Mg/35 ºC) the Mg concentrations in leaves increased from the 10th day and remained elevated until the end of the experiment. Even Mg concentration in leaves being within the adequate concentration for coffee, leaf chlorosis was observed from the 20th day.

On the other hand, Mg concentrations in leaves of seedlings growing with the exclusion of Mg at 25 ºC (-Mg/25 ºC) began to fall below the optimal level at the 20th day of evaluation, when the symptoms appeared (Table 1). Finally, seedlings cultivated in the presence of both stresses (-Mg/35 ºC) showed early symptoms of Mg deficiency at 10th day, when Mg leaf concentrations decreased significantly in relation to other treatments.

Regarding roots, both Mg deficiency and heat were responsible for drops in Mg concentration (Table 1). In addition, in the last evaluation, seedlings submitted to a combination of these stresses (-Mg/35ºC) had the lowest concentrations of magnesium when compared to other treatments.

Leaf levels of total chlorophyll were negatively impacted by both Mg exclusion and temperature rise (Table 1). On the 30th day, Mg-deficient seedlings, grown at 25 °C showed a reduction of 20 % in chlorophyll concentration when compared to seedlings well supplied in Mg. In addition, the increase in temperature led to a reduction of 71% in chlorophyll concentration in seedlings with Mg. Thus, temperature rise was responsible for a much greater drop in chlorophyll levels. In addition, the combination of stresses (-Mg/35 ºC) at the 30th day was responsible for the lowest chlorophyll concentrations among all treatments.

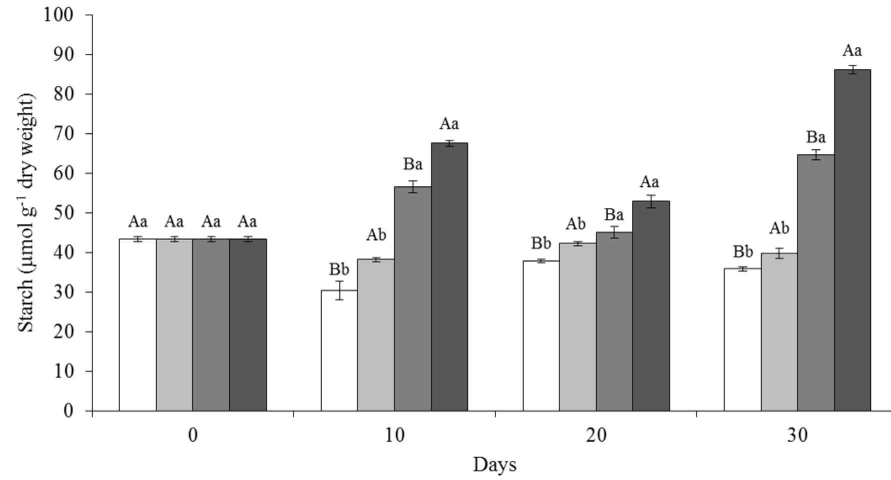
Table 1: Concentration of magnesium in leaves and roots and concentration of total chlorophyll in leaves of *Coffea arabica* L. seedlings submitted to different concentrations of magnesium and temperatures. Capital letters compare the effect of magnesium deficiency on each temperature and sampling time and lowercase letters compare the effect of temperature elevation on each Mg condition and sampling time. Different letters indicate significant differences with 0.05 probability. Bars show the standard error of five replicates.

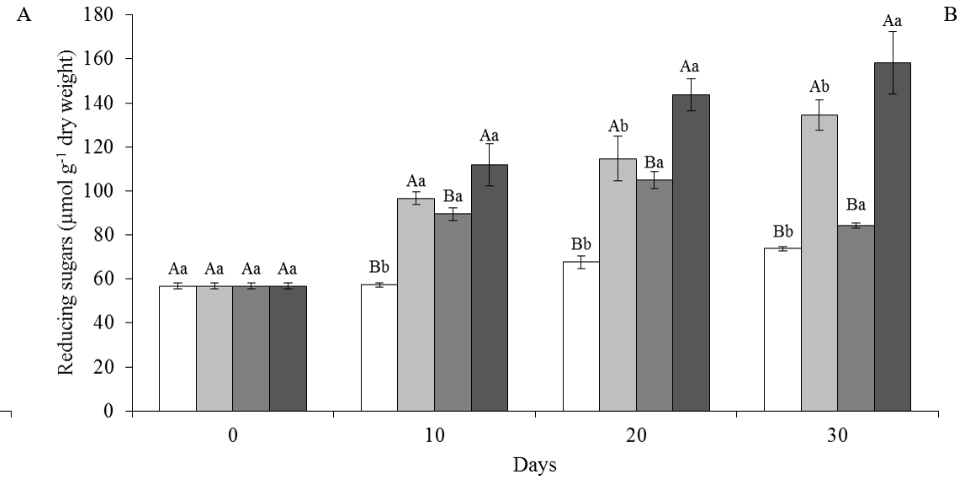
T. (ºC)	Days	Treat.	Shoot Mg concentration (mg Kg^{-1} DW)	Root Mg concentration (mg Kg^{-1} DW)	Total chlorophyll concentration (µg g^{-1} FW)
25	0	+Mg	3.5 Aa	3.7 Aa	1262 Aa
	10		3.6 Ab	4.0 Aa	1419 Aa
	20		3.3 Ab	4.2 Aa	1387 Aa
	30		3.4 Ab	3.9 Aa	1499 Aa
	0	-Mg	3.5 Aa	3.7 Aa	1262 Aa
	10		3.2 Ba	3.8 Aa	1140 Ba
	20		3.0 Ba	3.4 Ba	1256 Ba
	30		2.7 Ba	3.1 Ba	1204 Ba
35	0	+Mg	3.5 Aa	3.7 Aa	1262 Aa
	10		3.9 Aa	4.1 Aa	674 Ab
	20		3.7 Aa	4.0 Ab	528 Ab
	30		4.2 Aa	3.7 Ab	428 Ab
	0	-Mg	3.5 Aa	3.7 Aa	1262 Aa
	10		2.8 Bb	3.8 Ba	616 Bb
	20		2.6 Bb	3.2 Bb	437 Bb
	30		2.5 Bb	2.9 Bb	180 Bb

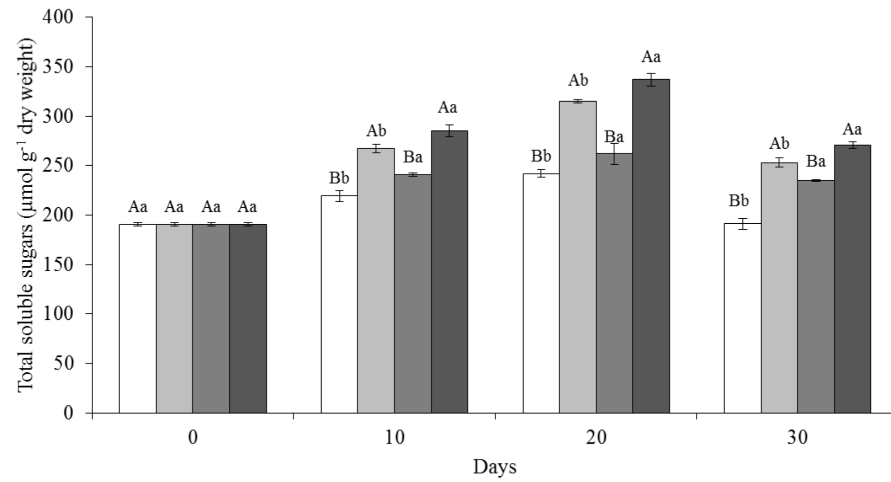
T.: Temperature; Treat.: Treatments; +Mg: Control; -Mg: Mg-deficient; DW: dry weight; FW: fresh weight.

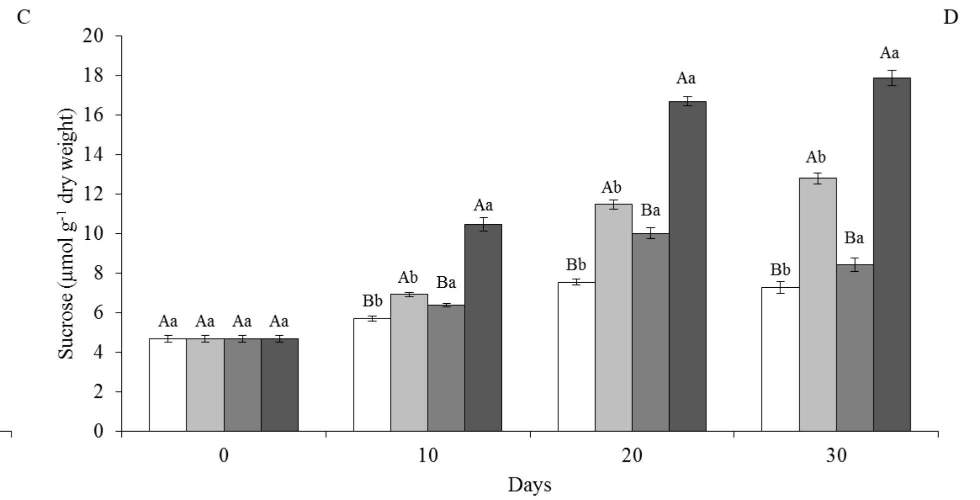
Magnesium exclusion and temperature rise were responsible for increases in leaf concentration of photoassimilates (Figure 2). At the 30th day, Mg deficiency in seedlings submitted to 25 ºC resulted in an increase of 11, 82, 32 and 76% in the leaf concentration of starch, reducing sugars, total soluble sugars and sucrose, respectively. Already the temperature rise in seedlings with Mg resulted in an increase of 80, 14, 23 and 16% in the levels of these carbohydrates. Thus, we have that temperature rise was responsible for an increase in leaf concentration of starch higher than that caused by Mg deficiency (Figure 2A). While for reducing sugars, total soluble sugars and sucrose the increase of their concentrations due to Mg deficiency was higher to that triggered by temperature rise

(Figure 2B, 2C and 2D). In addition, the combination of Mg deficiency and temperature rise culminated in the highest leaf levels of photoassimilates when compared to the other treatments.

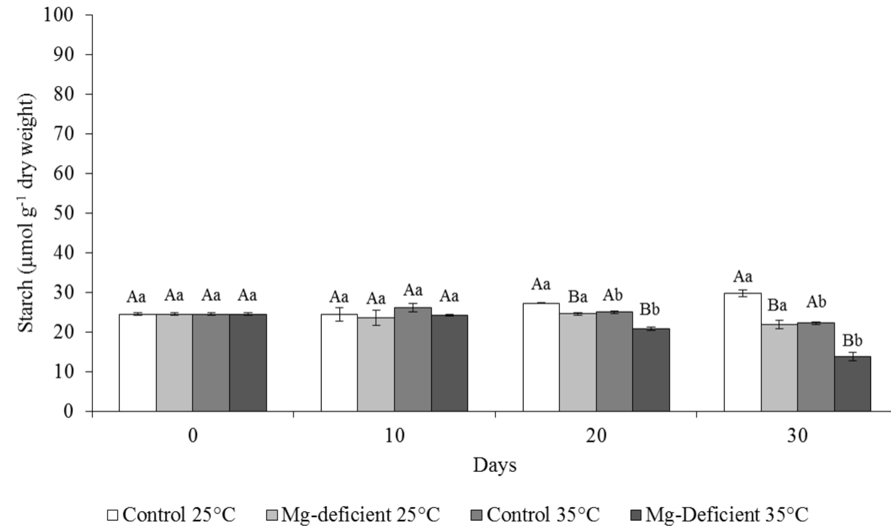

A
Starch (µmol g-1 dry weight)
Days
Control 25°C
Mg-deficient 25°C
Control 35°C
Mg-Deficient 35°C

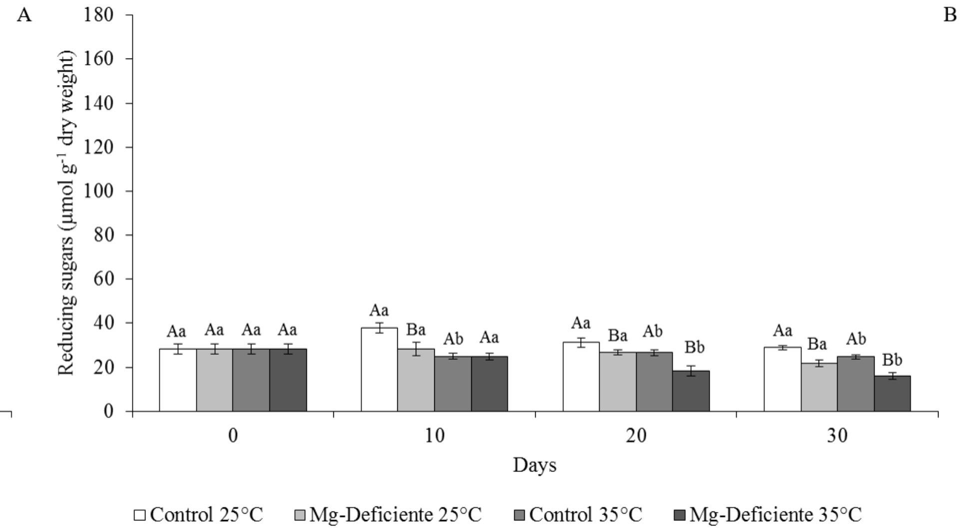

B
Reducing sugars (µmol g-1 dry weight)
Days
Control 25°C
Mg-Deficiente 25°C
Control 35°C
Mg-Deficiente 35°C

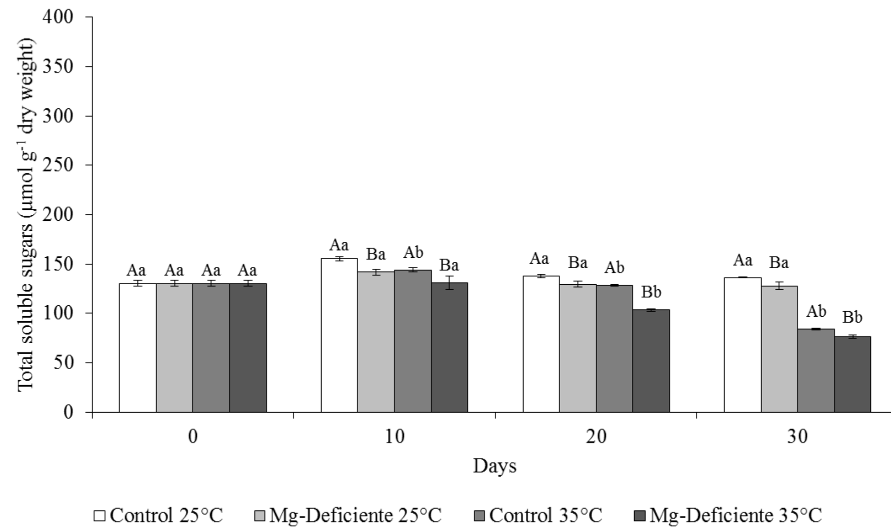

C
Total soluble sugars (µmol g-1 dry weight)
Days
Control 25°C
Mg-Deficiente 25°C
Control 35°C
Mg-Deficiente 35°C


D
Sucrose (µmol g-1 dry weight)
Days
Control 25°C
Mg-Deficiente 25°C
Control 35°C
Mg-Deficiente 35°C

1 Figure 2: Concentration of starch (A), reducing sugars (B), total soluble sugars (C) and
2 sucrose (D) in leaves of *Coffea arabica* L. seedlings submitted to different concentrations
3 of magnesium and temperatures. Capital letters compare the effect of magnesium
4 deficiency on each temperature and sampling time and lowercase letters compare the
5 effect of temperature elevation on each Mg condition and sampling time. Different letters
6 indicate significant differences with 0.05 probability. Bars show the error of five
7 replicates.
8
9 Mg deficiency and heat triggered reductions in the concentration of
10 photoassimilates in roots (Figure 3). In the last evaluation, the drop in root concentration
11 of starch, reducing sugars, total soluble sugars and sucrose due to Mg exclusion was 26,
12 25, 6 and 39%, respectively. Temperature rise, in turn, was responsible for a drop of 25,
13 14, 38 and 21% in the levels of these carbohydrates. Thus, it was observed that
14 temperature rise had an impact on the root concentration of reducing sugars and total
15 soluble sugars higher than that observed in seedlings submitted to Mg exclusion (Figure
16 3B and 3C). However, Mg deficiency resulted in greater decrease in sucrose levels than
17 temperature rise (Figure 3D). Despite these differences, both Mg deficiency and heat were
18 responsible for reducing the translocation of photoassimilates from shoots to roots.
19 Moreover, the combination of these stresses (-Mg/35 ºC) was responsible for reaching the
20 lowest root levels of photoassimilates when compared to other treatments.
21


A
Starch (µmol g-1 dry weight)
Days
Control 25°C
Mg-deficient 25°C
Control 35°C
Mg-Deficient 35°C


B
Reducing sugars (µmol g-1 dry weight)
Days
Control 25°C
Mg-Deficiente 25°C
Control 35°C
Mg-Deficiente 35°C


C
Total soluble sugars (µmol g-1 dry weight)
Days
Control 25°C
Mg-Deficiente 25°C
Control 35°C
Mg-Deficiente 35°C

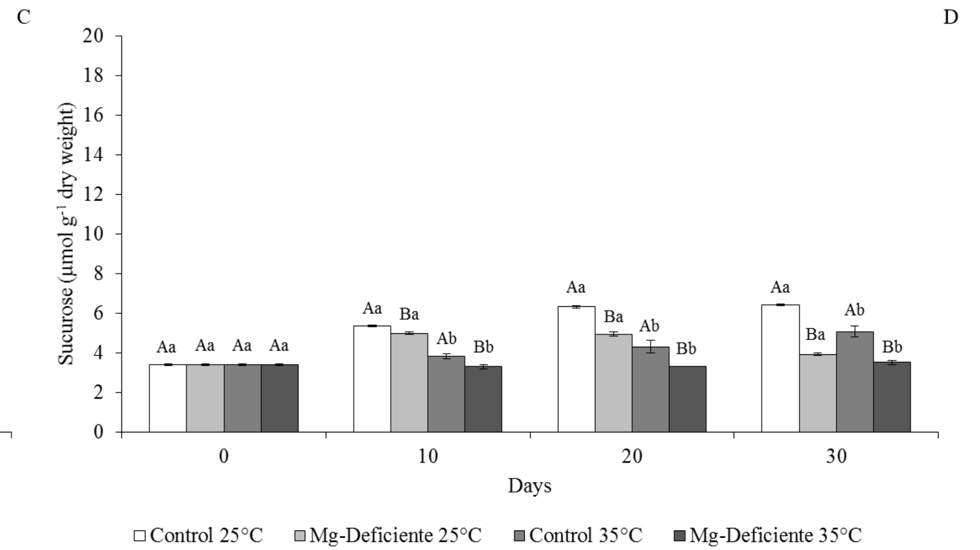

D
Sucurose (µmol g-1 dry weight)
Days
Control 25°C
Mg-Deficiente 25°C
Control 35°C
Mg-Deficiente 35°C

Figure 3: Concentration of starch (A), reducing sugars (B), total soluble sugars (C) and sucrose (D) in roots of *Coffea arabica* L. seedlings submitted to different concentrations of magnesium and temperatures. Capital letters compare the effect of magnesium deficiency on each temperature and sampling time and lowercase letters compare the effect of temperature elevation on each Mg condition and sampling time. Different letters indicate significant differences with 0.05 probability. Bars show the standard error of five replicates.

The activity of acidic cell wall (IAPC), vacuole acid (IAV) and neutral cytosol invertases (INC) in leaves was reduced by both Mg exclusion and temperature rise (Table 2). At day 30, Mg deficiency alone led to a drop of 59, 24 and 79% in the leaf activity of these enzymes, respectively. While the temperature rise culminated in a reduction of 54, 34, and 34% in the leaf activity of this enzymes. Therefore, Mg-deficiency and heat had a very similar impact on the IAPC and IAV leaf activity (Figure 5A and 5B). However, for INC, the exclusion of Mg had a greater impact on its activity than temperature rise (Table 2). Finally, leaves of seedlings exposed to the combination of these stresses (-Mg/35 ºC) showed the lowest activities of these enzymes when compared to the other treatments.

The combination of stresses, Mg deficiency and temperature rise, culminated in the lower activities of the invertases in roots of *Coffea arabica* L. seedlings (Table 2). In the last evaluation, exclusion of Mg alone resulted in a decrease in IAPC, IAV and INC activities of 65, 68 and 51%, respectively. Heat, on the other hand, was responsible for the 25, 46 and 48% reduction in the activity of these enzymes. Thus, we have that Mg deficiency resulted in a greater drop in IAPC and IAV activity of roots than temperature rise. For INC, however, the reduction was similar for both stress.

Table 2: Acid cell wall (IAPC), vacuole acid (IAV) and neutral cytosol (INC) invertases activities in shoots and roots of *Coffea arabica* L. seedlings submitted to different concentrations of magnesium and temperatures. Capital letters compare the effect of magnesium deficiency on each temperature and sampling time and lowercase letters compare the effect of temperature elevation on each Mg condition and sampling time. Different letters indicate significant differences with 0.05 probability. Bars show the standard error of five replicates.

T (ºC)	Days	Treat.	IAPC Leaf	IAPC Root	IAV Leaf	IAV Root	INC Leaf	INC Root
			(µmol glucose min^{-1} mg^{-1} fresh weight)					
25	0	+ Mg	0.18 Aa	0.06 Aa	0.17 Aa	0.06 Aa	0.22 Aa	0.09 Aa
	10		0.30 Aa	0.10 Aa	0.25 Aa	0.07 Aa	0.27 Aa	0.09 Aa
	20		0.32 Aa	0.08 Aa	0.23 Aa	0.06 Aa	0.27 Aa	0.11 Aa
	30		0.24 Aa	0.08 Aa	0.25 Aa	0.08 Aa	0.37 Aa	0.13 Aa
	0	- Mg	0.18 Aa	0.06 Aa	0.17 Aa	0.06 Aa	0.22 Aa	0.09 Aa
	10		0.28 Aa	0.07 Ba	0.15 Ba	0.03 Ba	0.13 Ba	0.08 Ba
	20		0.17 Ba	0.04 Ba	0.17 Ba	0.02 Bb	0.07 Ba	0.07 Ba
	30		0.10 Ba	0.03 Ba	0.19 Ba	0.03 Ba	0.08 Ba	0.06 Ba
35	0	+ Mg	0.18 Aa	0.06 Aa	0.17 Aa	0.06 Aa	0.22 Aa	0.09 Aa
	10		0.16 Ab	0.08 Aa	0.17 Ab	0.07 Aa	0.19 Ab	0.08 Aa
	20		0.13 Ab	0.06 Ab	0.17 Ab	0.07 Aa	0.17 Ab	0.07 Ab
	30		0.11 Ab	0.06 Ab	0.17 Ab	0.04 Ab	0.24 Ab	0.07 Ab
	0	- Mg	0.18 Aa	0.06 Aa	0.17 Aa	0.06 Aa	0.22 Aa	0.09 Aa
	10		0.08 Bb	0.06 Ba	0.07 Bb	0.02 Ba	0.09 Ba	0.04 Bb
	20		0.05 Bb	0.02 Bb	0.08 Bb	0.03 Ba	0.10 Ba	0.03 Bb
	30		0.04 Bb	0.01 Bb	0.08 Bb	0.03 Ba	0.08 Ba	0.04 Bb

T.: Temperature; Treat.: Treatments; +Mg: Control; -Mg: Mg-deficient.

Discussion

Deficiency of any mineral essential to plant life significantly reduces its growth and development. When associated with other types of stress, such as high temperature, for example, this reduction in dry weight is commonly aggravated. As expected, the interaction between Mg deficiency and heat stress was responsible for reaching the lowest dry weight values in shoot and roots (Figure 1), with root tissue being the most sensitive. When stresses were analyzed separately, the temperature rise from 25 to 35 °C imposed a greater impact on the accumulation of dry weight of leaves and roots than the exclusion of Mg. In addition, Mg exclusion and temperature rise affected the root system at a higher intensity than shoot.

Adequate Mg concentrations in coffee leaves usually range from 3.1 to 5.2 mg Kg^{-1} dry weight (Martinez et al. 2003, 2004). Thus, seedlings submitted simultaneously to Mg exclusion and to heat stress showed Mg levels in leaves (Table 1) below the optimum already at the tenth day. On the other hand, in seedlings Mg-deficient and exposed at 25 ºC levels below the optimum were reached only on the twentieth day. Thus, magnesium deficiency in seedlings of *Coffea arabica* L. cv. Catuaí has been aggravated and anticipated in approximately ten days by temperature rise.

Oriza sativa plants submitted to Mg deficiency showed a reduction in transpiration stream and a consequent decline in mineral absorption (Kobayashi et al. 2013). The mechanisms involved in this process are still unknown; however, it is believed that there is a signaling cascade mediated by abscisic acid (Kobayashi et al. 2013). In *Arabidopsis thaliana*, the levels of ABA-responsive gene expression were altered within the first 28 hours of exposure to magnesium deficiency (Hermans et al. 2010). In addition, problems in transpiration process may theoretically increase plant susceptibility to heat by preventing water evaporation that would promote foliar cooling (Mengutay et al. 2013). Therefore, the combination of stresses (-Mg/35 ºC) would have a major impact mainly on leaf tissue due to elevated temperature in leaf tissue.

On the other hand, heat alone triggers an increase in transpiratory rates of 4 to 6 times in coffee plants. This increase in transpiration stream results in greater absorption and translocation of mineral nutrients such as nitrogen, potassium, calcium and magnesium (Martins et al. 2014). Thus, elevation of transpiration flow, due to the elevation of temperature, may have triggered a greater translocation of nutrients from roots to shoots, causing greater Mg concentration in leaves of seedlings with Mg and submitted to 35ºC (Table 1) than those exposed at 25 °C. Therefore, roots of seedlings with Mg when submitted to 35 ºC showed lower values of Mg (Table 1) than those of seedlings exposed at 25 ºC.

One of the major roles of magnesium in plant physiology is the participation as the central atom of chlorophyll molecule. In this way, Mg-deficiency in plants of *Morus alba* L. (Tewari; Kumar; Sharma 2006), *Sulla carnosa* (Farhat et al. 2014) and *Coffea arabica* L. (Silva et al. 2014) was responsible for a reduction on chlorophyll levels. Coffee seedlings showed substantial reductions in total chlorophyll concentration (Table1) due to both Mg deficiency and temperature rise. However, more pronounced reductions were observed because of temperature rise. Combination of these stresses (-Mg/35 ºC) was responsible for achieving the lowest levels of total chlorophyll among other treatments, which may have occurred due to the combination of the isolated effects of each stress.

The reduction in total chlorophyll concentration in Mg-deficient plants has been attributed to leaf accumulation of sugars, rather than to the reduction of Mg levels (Cakmak; Hengeler; Marschner 1994a, b). This is because the accumulation of photoassimilates in leaves can negatively regulate genes involved in photosynthesis, including those that encode chlorophyll-binding proteins (Hermans et al. 2004). In coffee seedlings a low correlation between Mg concentration and total chlorophyll levels was observed, whereas correlations of -0.86 and -0.60 were observed between leaf concentration of starch and sucrose and chlorophyll levels, respectively. Thus, it can be suggested that the decrease in chlorophyll concentration (Table 1), due to both magnesium deficiency and elevated temperature, is more related to the negative feedback of carbon metabolism on chlorophyll biosynthesis than to Mg concentration in leaves. In addition, it can also be ruled out that the temperature rise can cause changes in thylakoid structure, inactivation of enzymes of chlorophyll biosynthesis pathway and degradation of photosynthetic pigments (Dutta and Mohanty 2009). Thus, the reduction in chlorophyll concentration due to heat can occur in one or both ways (Mathur; Agrawal; Jajoo 2014).

As previously observed by SILVA et al. (2014), *Coffea arabica* L. seedlings Mg-deficient (-Mg/25 °C) showed increases in leaf concentration of starch, reducing sugars, total soluble sugars and sucrose (Figure 2), as well as reductions in their concentrations in roots (Figure 3), when compared to seedlings with Mg (+Mg/25 °C). The causes of sucrose accumulation in source leaves may be due to the reduction in metabolic activity of sink organs (Fischer et al. 1998), or by impairment of phloem loading (Gransee and Fhürs 2013, Mengutay et al. 2013).

Sucrose loading is an active process catalyzed by the co-transport of H^+ and sucrose, which involves a proton gradient through plasma membrane of the phloem cells. The proton gradient required for co-transport of H^+ and sucrose is established by an H^+-ATPase, located on plasma membrane of sieve tube cells (Ward et al. 1998). The proper functioning of the H^+-ATPase (Getz and Klein 1995) is related to Mg-ATP, which is the main ATP complex in biological systems (Igamberdiev and Kleczkowski 2003). Therefore, a decrease in Mg-ATP concentration at phloem loading sites is more likely to be the main reason for the inhibition of the sucrose transport of Mg-deficient leaves (Cakmak and Kirby 2008).

In coffee seedlings a correlation of -0.75 was observed between the Mg and sucrose concentrations in leaves, suggesting that the accumulation of sucrose in leaves can be related to the reduction in Mg availability. Therefore, the reduction in the translocation of sucrose from leaves to roots in seedlings Mg-deficient can be associated with a problem in process of sucrose

loading into phloem. Similar results were observed in *Arabidopsis thaliana* (Hermans and Verbruggen 2005), *Triticum aestivum* and *Zea mays* (Mengutay et al. 2013) and *Coffea arabica* L. (Silva et al. 2014).

Regarding the impacts caused by the elevation of temperature, we observed an increase in the carbohydrate concentration in leaves (Figure 2), as well as a reduction in roots (Figure 3), similar results to those observed in seedling Mg-deficient. Previous works have shown that carbohydrate translocation from leaves to roots is reduced when plants are submitted to temperature rise in different plant species (Timlin et al. 2006; Huang; Rachmilevitch; Xu 2012). However, mechanisms involved in disturbing on carbohydrates translocation from source organs to sink organs due to temperature rise remain unknown.

Moreover, carbon loss through respiration is increased in roots when submitted to temperature rise (Huang; Rachmilevitch; Xu 2012), which would also explain the lower carbohydrates concentration in roots of seedlings subjected to heat when compared to those grown at 25 °C. Thus, the low carbohydrate levels found in roots of seedlings submitted to heat stress can be associated with the reduction in photoassimilates translocation to roots and the increase in roots respiratory rates (Huang; Rachmilevitch; Xu 2012).

Since Mg deficiency also reduces root concentration of carbohydrates, it can be suggested that the adverse impacts of heat stress on roots may be more pronounced when plants are simultaneously exposed to heat stress and Mg deficiency. Thus, greater attention should be given to the nutritional status of plants, especially regarding magnesium nutrition, since the importance of a good root system for plant growth and development.

For a better understanding of the carbohydrate partitioning process, the activity of invertases was analyzed. Negative correlations were observed between leaf activities of acidic cell wall and vacuole and neutral cytosol invertases and leaf concentrations of reducing sugars and sucrose. The reduction in leaf activity of invertases (Table 2) may have occurred by a negative feedback mechanism due to photoassimilates accumulation in leaves (Figure 2). This can happen because simple sugars, such as glucose and sucrose, are modulators of the expression of the genes that encode invertases synthesis (Brocard-Gifford; Lynch; Finkelstein 2001).

The transport of photoassimilates from source to sink organs is driven by differences in potentials along phloem (Kock 2004). Sucrose loading on phloem increases the sucrose concentration on phloem triggering a reduction in osmotic potential. With lower osmotic potential on phloem in relation to xylem, water is transported to xylem from phloem, triggering a rise in pressure potential on phloem (KOCH, 2004). On the other hand, sucrose downloading

in sink organs triggers an increase on osmotic potential of phloem and water is lost to xylem leading to a low pressure potential. In this way, a sucrose tends to be transported by mass flow from a region with high pressure potential to a low pressure potential, ie from source to sink. Thus, the cleavage of sucrose by invertases at phloem downloading sites controls the force of sink in attracting more sucrose (Eschrich 1980). Temperature rise and magnesium deficiency triggered a reduction in invertase activity in roots of coffee seedlings. Thus, the osmotic potential of phloem in proximal region of roots was probably increased due to accumulation of sucrose, resulting in a

reduction of sink force.

As expected, dry weight accumulation of *Coffea arabica* L. seedlings was affected by magnesium deficiency. In addition, root growth was more sensitive to Mg deficiency, resulting in a higher shoot:root dry weight ratio (Figure 1). More severe reductions in root growth due to Mg deficiency have also been reported in *Phaseolus vulgaris* (Cakmak; Hengeler; Marschner 1994a), *Citrus* sp. (Yang et al. 2012), *Triticum aestivum* and *Zea mays* (Mengutay et al. 2013) and *Coffea arabica* L. cv. Catuaí and Acaiá (Silva et al. 2014).

Cakmak, Hengeler and Marschner (1994b) working with *Phaesolus vulgaris* submitted to Mg deficiency observed that there is a clear relation between shoot:root dry weight ratio and the relative carbohydrates distribution between shoot and root. In coffee seedlings submitted to Mg deficiency, carbohydrate concentrations increased in leaves (Figure 2) and reduced in roots (Figure 3). This fact, together with high shoot:root dry weight ratio (Figure 1), suggests a decrease in photoassimilates exportation from leaves to roots (Cakmak; Hengeler; Marschner 1994a). However, the reasons for this reduction in carbohydrate partitioning are still unknown. It is suggested that the problem is related to the reduction on sink force or due to sucrose loading process into phloem.

Greater susceptibility of roots to heat stress was also observed in species such as *Agrostis palustris* (Huang and Gao 2000), *Picea mariana* (Way and Sage 2008), *Triticum aestivum* and *Zea mays* (Mengutay et al. 2013). The lower translocation of carbohydrates to roots may be related to this greater susceptibility. The reduction in carbohydrate partitioning due to temperature rise was observed in *Coffea arabica* L. seedlings (Figures 2 and 3), as well as previously reported in *Solanum tuberosum* L. (Timlin et al. 2006) and *Agrostis* sp (Huang; Rachmilevitch; Xu 2012). In addition, a correlation of 0.87 was observed between root concentration of sucrose and the dry weight accumulation of this organ, corroborating with the idea that the reduction in photoassimilates concentration in roots by both magnesium and heat stress is, in part, responsible for root growth impairment.

In addition, carbon loss via root respiration is higher under conditions of temperature rise (Huang; Rachmilevitch; Xu 2012). Thus, the low carbohydrate levels in roots of seedlings submitted to heat stress can be both associated to the reduction in photoassimilates translocation to roots, as well as to the increase in root respiratory rates, which resulted in a lower accumulation of dry weight in this organ.

Heat stress is a growing concern in the production of many crops due to the current global warming. Mg deficiency, in turn, has become an important nutritional deficiency in agricultural soils. Particularly in *Coffea arabica* L., since coffee crops has been implemented at agricultural frontiers, where soils are acid, with low cation exchange capacity and poor in magnesium. In *Coffea arabica* L., effects of temperature rise on plant metabolism are more pronounced when seedlings are simultaneously exposed to Mg deficiency, mainly the reduction in photoassimilates translocation to roots and root growth, as well as the remarkable increase in shoot:root dry weight ratio.

In this way, proper nutrition in Mg is a good strategy to minimize losses related to temperature rise in production of coffee crops. This can be considered not only for vegetative growth, but also for the time of grain filling, when both the temperature rise and Mg deficiency would have a strong impact on grain filling, mainly by the impairment of photoassimilates exportation. Thus, greater attention is needed on magnesium nutrition in *Coffea arabica* L. seedlings cv. Catuaí to mitigate losses due to temperature rise.

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Artigo 2 - Adequate Mg nutrition is essential for an efficient antioxidant response in coffee seedlings under heat stress

Artigo apresentado nas normas da revista Scientia Horticulturae (Versão submetida em 26 de janeiro de 2017)

Abstract

High temperatures already are a reality of current days, if necessary attention is not given to plant nutrition, including with regard to magnesium nutrition, large losses in productivity can occur when plants are submitted to a combination of these stresses. Thus, the aim of our work was to investigate the importance of adequate magnesium nutrition for an efficient antioxidant response in coffee seedlings submitted to heat stress. Then, six months old seedlings of *Coffea arabica* L. were transferred to plastic containers containing a nutrient solutions with different concentrations of magnesium (Mg) and two different temperatures (25 and 35 °C). Fully expanded leaves and roots were evaluated at the beginning of treatment and after 10, 20 and 30 days for hydrogen peroxide, proline, ascorbate, malondialdehyde, protein and amino acid concentration, and activities of superoxide dismutase, catalase, ascorbate peroxidase, monodehydroascorbate reductase, dehydroascorbate reductase and glutathione reductase. Variables analyzed were mainly affected by combination of stresses. Mg-deficiency and heat stress caused an increase in hydrogen peroxide concentration that was accompanied by a rise in antioxidant metabolism and by greater production of proline and ascorbate. Nevertheless, antioxidant metabolism and osmoprotectant were insufficient to remove ROS excess, resulting in increased lipid peroxidation and protein degradation. When submitted to heat stress, coffee seedlings under adequate Mg nutrition showed lower production of hydrogen peroxide and consequently lower lipid peroxidation and protein denaturation than those deficient in Mg and submitted to heat stress. Therefore, an adequate Mg nutrition is essential to minimize oxidative damage caused by heat stress in coffee seedlings.

Keywords hydrogen peroxide, ascorbate-glutathione cycle, oxidative stress

Introduction

Heat stress has become a growing concern due to damage caused to productivity of many crops (Luo, Li and Wang 2014). Increases in temperature trigger impacts in several

physiological and metabolic processes, such as carbohydrates partitioning to root and consequent damage to root growth (Du and Tachibana 1994). Other stress that also cause this reduction in carbohydrate translocation from source to sink is magnesium deficiency (Cakmak and Yazici 2010; Silva et al. 2014). High carbohydrate concentration in leaf tissue exposes chloroplasts to an excessive excitation energy triggering high production of reactive oxygen species (ROS), such as superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (OH^-) and singlet oxygen ($^1{}_2O$) (Marutani et al. 2012).

Once ROS are unavoidable products of plant metabolism, under normal conditions ROS production and removal are well balanced (Mittler 2002). However, under stress conditions, ROS production can overcome removal mechanisms triggering oxidative stress (Marutani et al. 2012). This is due to the high toxicity of ROS, which are highly cytotoxic and can react with various biomolecules, such as lipids, proteins and nucleic acids, causing lipid peroxidation, protein denaturation and DNA mutations, respectively (Gill and Tuteja 2010).

Membranes are the main target of injury, since ROS can react with unsaturated fatty acids triggering lipid peroxidation in plasma membranes and in organelles membranes (Sharma et al. 2012). Peroxidation of plasma membrane cause extravasation of cellular content, rapid desiccation and cell death, whereas in intracellular membrane can affect mitochondrial respiratory activity, pigment deterioration and loss of carbon fixation capacity in chloroplast (Gill and Tuteja, 2010).

To counteract the effects of heat on cellular metabolism, plants respond by synthesizing and accumulating osmoprotectants. They are small and electrically neutral molecules that stabilize proteins and membranes against the denaturant effects of heat (Rivero et al. 2014). Proline is one of osmoprotectant that is responsible by stabilization of proteins, membranes and subcellular structures, and by removal of reactive oxygen species (Kumar et al. 2012).

In addition to osmoprotectants, to protect cells from oxidative damage, plants have developed different antioxidant defense systems to minimize ROS cellular damage. Enzymatic antioxidant system includes superoxide dismutase (SOD), which catalyzes the dismutation of O_2^- to H_2O_2 and O_2. H_2O_2 is converted to H_2O and O_2 by action of catalases (CAT), various peroxidases and by enzymes of the ascorbate-glutathione cycle. This cycle is characterized by a series of coupled redox reactions, involving ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), glutathione reductase (GR) and ascorbate (Gill and Tuteja, 2010).

High temperatures already are a reality of current days, if necessary attention is not given to plant nutrition, including with regard to magnesium nutrition, large losses in

productivity can occur when plants are submitted to a combination of these stresses. Manly for coffee plants, since this crop has been implemented in Brazil's agricultural frontiers (Tomaz et al., 2003), where soils are acid, have low cation exchange capacity and are poor in magnesium. Thus, the aim of our work was to investigate the importance of adequate magnesium nutrition for an efficient antioxidant response in coffee seedlings submitted to heat stress.

Material and methods

Plant culture and Mg treatments

Coffea arabica L. seedlings from Catuaí 144 cultivars were cultivated for six months in polypropylene bags of 500 mL filled with subsoil and cattle manure in 2:1 proportion, plus potassium chloride and superphosphate in the proportion 1:10 (Guimarães et al. 2002). After selection for uniformity in size and vigor, seedlings were transferred to 40 L plastic containers (19x43x66 - HxWxL) containing a nutrient solution (Hoagland and Arnon 1950). Each recipient contained 16 plants. The experiment was conducted in a growth room, with photosynthetic photon flux density around 250 μmol m^{-2} s^{-1} and relative humidity of 50% and 60% during light and dark periods, respectively.

Plants were acclimated for 6 days when solutions with increasing concentrations were used: ¼ strength for 2 days, ½ strength for 2 days, and full strength for 2 days. After six days of acclimation, plants were submitted to four treatments: a) complete nutrient solution with the original concentration of Mg and air temperature of 25 °C (+Mg 25 °C); B) nutrient solution with exclusion of Mg and air temperature of 25 °C (-Mg 25 °C); c) complete nutrient solution with the original concentration of Mg and air temperature of 35 °C (+Mg 35 °C) and d) nutrient solution with exclusion of Mg and air temperature of 35 °C (-Mg 35 °C).

The volume of the nutrient solution was daily replenished with deionized water. The pH of the solution was also adjusted daily to 5.5 ± 0.5 with NaOH and HCL solution (1 mol L^{-1}). The solutions were replaced weekly. All seedlings were maintained under constant aeration throughout the experimental period. The temperature in the root environment during the completely experimental period was 3 °C lower than that of the leaf environment; and the night temperature was 5 °C below the diurnal temperature, both in the leaf environment and in the root environment.

The design was completely randomized, in a 2x2 factorial scheme, with two treatments (with and without Mg) and two temperatures (25 °C and 35 °C), with five replications. At 0,

10, 20 and 30 days after the imposition of the treatments, completely expanded leaves of the third and fourth pairs and the root system of five plants were collected. Then, plant material was conditioned in liquid nitrogen and stored at -80 °C for analyzes on fresh weight, or brought to the oven at 70 °C under forced circulation to constant weight for analyzes on dry weight.

Variables were analyzed for normality by the Shapiro Wilk Test ($p \geq 0.05$). The variables that fit within the normal range were submitted to analysis of variance, using the SISVAR 4.3 statistical system (Variance Analysis System for Balanced Data) (Ferreira 2011), where the means between the treatments were compared by the Scott test -Knott at 0.05 probability. The variables that did not fit into the normality were transformed by Box-Cox Method and subjected to analysis of variance and mean test by Scott-Knott at 0.05 probability. The non-parametric variables were analyzed by the Kruskal-Wallis Method at 0.05 probability. Pearson correlations were performed between the variables using the statistical program Action Stat (Equipe Estatcamp 2014).

Antioxidant metabolism

H_2O_2 was determined according Velikova et al. (2000): 200 mg of fresh weight were macerated in liquid nitrogen, added to polyvinylpolypyrrolidone (PVPP) and homogenized in 1500 μL of 0.1% trichloroacetic acid (TCA) (m/v). The homogenate was centrifuged at 12,000 g for 15 minutes at 4 °C. H_2O_2 was determined by measuring the absorbance at 390 nm in a reaction medium containing 45 μL of extract, 45 μL of 10 mM potassium phosphate buffer (pH 7.0) and 90 μL of 1M potassium iodide.

Extraction and quantification of proline were made by method of Bates, Waldren and Teare (1973). It was carried out by maceration of 100 mg of dried weight with 3% sulfosalicylic acid. The extracts were stirred at room temperature for 60 minutes and filtered with filter paper. Aliquots of filtrate reacted with 2 ml acid ninhdrin and 2 ml of glacial acetic acid in a test tube for 1 hour at 100°C, and the reaction terminated in an ice bath. Quantification was performed based on the standard curve with increasing and known concentrations of proline.

SOD, CAT, APX, MDHAR, DHAR and GR were extracted according Biemelt, Keetman and Albrecht (1998): 0,2 g of fresh weight were macerated in liquid nitrogen and PVPP and homogenized in 1,5 mL of extraction buffer containing: 400 mM potassium phosphate buffer (pH 7.8), 10 mM EDTA, 200 mM ascorbic acid and water. The extract was centrifuged at 13,000 g for 10 minutes at 4 ° C and the supernatant was collected and stored at -20 ° C during the analysis period.

SOD activity was measured by the ability of enzyme to inhibit the photoreduction of nitroblue tetrazolium (NBT) (Giannopolitis; Ries, 1977). Aliquots (10 µL) of enzyme extract were added to incubation medium containing 100 mM potassium phosphate (pH 7.8), 70 mM methionine, EDTA 10 µM, water, 1 mM NBT and 0.2 mM riboflavine. Tubes containing the reaction medium and 10 µL of sample were illuminated for 7 minutes with a fluorescent lamp of 20 W. The same reaction medium without a sample was illuminated as a control. One unit of SOD is able to inhibit 50% of the photoreduction of NBT under the assay conditions.

CAT was evaluated according Mengutay et al. (2013): aliquots of enzyme extract were added to incubation medium containing 50 mM potassium phosphate buffer (pH 7.6), 0,1 mM disodium ethylenediamine tetraacetic acid (Na_2EDTA), water and 200 mM hydrogen peroxide, incubated at 30 °C. Enzyme activity was determined by the decrease in absorbance at 240 nm every 15 seconds for 3 minutes, monitored by the consumption of hydrogen peroxide. The molar extinction coefficient used was 36 mM^{-1} cm^{-1}.

APX activity was determined by monitoring of the rate of oxidation of ascorbate at 290 nm every 15 seconds for 3 minutes. Aliquots of enzyme extract were added to an incubation buffer, consisting 100 mM potassium phosphate buffer (pH 6.0), 10 mM ascorbic acid, water and 10 mM hydrogen peroxide (Nakano, Asada, 1981). The molar extinction coefficient was 2.8 mM^{-1} cm^{-1}.

Ascorbate concentration was determined as described by Arakawa et al. (1981): 50 mg of fresh weight were macerated in liquid nitrogen, added to PVPP and homogenized in 1500 µL of trichloroacetic acid (TCA) 5% (m/v). The homogenate was then centrifuged at 13,000 g for 15 minutes at 4 °C. Aliquots of the supernatant were added to the reaction medium composed of TCA 5% (m/v), ethanol 99,8% (v/v), phosphoric acid (H_3PO_4) 0,4% in ethanol (v/v), bathophenantrolina 0,5% ethanol (m/v) and ferric chloride ($FeCl_3$) 0,03% in ethanol (m/v). The mix was homogenized thoroughly and incubated at 30 °C for 90 minutes. Readings were performed at 534 nm.

Monodehydroascorbate reductase (MDHAR) was assayed according to a method following Hossain et al. (1984). Each reaction mixture contained 100 mM tris hydrochloride (Tris-HCl) buffer (pH 7.6), 10 mM ascorbic acid, 4 mM nicotinamide adenine dinucleotide (NADH) and water. The reaction was followed by measuring the decrease in absorbance at 340 nm due to NADH oxidation.

The assay of dehydroascorbate reductase (DHAR) activity was carried out by measuring the increase in absorbance at 265 nm due to ascorbate formation (Nakano and Asada 1981).

The incubation mix contained 100 mM potassium phosphate (pH 7.0), 0.8 mM DHA 0.1 mM EDTA, water and 2.5 mM reduced glutathione (GSH).

Glutathione reductase (GR) activity was determined by monitoring of the rate of oxidation of nicotinamide adenine dinucleotide phosphate (NADPH) at 340 nm every 15 seconds for 3 minutes. Aliquots of enzyme extract were added to an incubation buffer, consisting 100 mM potassium phosphate buffer (pH 7.8), 10 mM oxidized glutathione, water and 5mM NADPH (Cakmak, Strbac and Marschner 1993).

Lipid peroxidation was determined by quantification of thiobarbituric acid reactive species, as described by Buege and Aust (1978). Two hundred milligrams of fresh weight were macerated in liquid nitrogen, added to PVPP (m/m) and homogenized in trichloroacetic acid (TCA) 0.1% (m/v). The homogenate was centrifuged at 10,000 g for 10 minutes at 4°C. Aliquots of the supernatant were added to the reaction medium [thiobarbituric acid (TBA) 0.5% (m/v) and TCA 10% (m/v)], and then incubated at 95°C for 30 minutes. Rapid cooling on ice stopped the reaction and readings were determined in a spectrophotometer at 535 nm and 600 nm. TBA form complexes of red color with low molecular weight aldehydes, such as malondialdehyde (MDA), a by-product of the peroxidation process. Concentration of MDA/TBA complex was calculated by the following equation: $[MDA] = (A_{535} - A_{600}) / (\xi.b)$, where: ξ (extinction coefficient = 1.56×10^{-5} cm^{-1}); b (optical length = 1).

Evaluation of protein and amino acid

The extraction of protein and amino acid was performed according to Zanandrea et al. (2010): 200 mg of dry weight were homogenized in 5 mL of potassium phosphate buffer 100 mM (pH 7.0) and then placed in a water bath for 30 minutes at 40 °C. Homogenate was centrifuged at 5,000 g for 10 minutes and the supernatant was collected. The process was repeated twice and supernatants were combined. Protein were quantified as described by Bradford (1976), and level of amino acid was quantified according to Yemm, Cocking and Ricketts (1955).

Results

Mg deficiency and heat stress resulted in increases in leaf and root production of H_2O_2 (Table 1). This increase started on 10th day in Mg-deficient seedlings exposed to 35 ºC and from 20th day for those at 25 ºC. At 30th day, the increase in H_2O_2 production in leaves (Table 1) due

to Mg exclusion and heat stress, alone, was of 88% and 78%, respectively. In roots, the increase in production of hydrogen peroxide was 49% in Mg-deficient seedlings and 24% in seedlings exposed to high temperature (Table 1). Finally, the combination of these stresses resulted in the highest levels of hydrogen peroxide than those observed in each stress alone.

Proline levels were also elevated by Mg exclusion and heat stress (Table 1). In the last evaluation, Mg deficiency, alone, resulted in an increase in proline concentrations of 37% in leaves and 32% in roots. In addition, temperature rise from 25 to 35 ° C resulted in a much larger increase in proline production. When submitted to heat stress, the increases in proline concentrations were of 324% in leaves and 385% in roots at the end of the experimental time. Moreover, when these stresses were combined, the greatest proline levels were observed.

Table 1: Concentrations of hydrogen peroxide, and proline in leaves and roots of *Coffea arabica* L. seedlings submitted to different concentrations of magnesium and temperatures. Capital letters compare the effect of magnesium deficiency on each temperature and sampling time and lowercase letters compare the effect of temperature elevation on each Mg condition and sampling time. Different letters indicate significant differences with 0.05 probability, according Scott-Knott test.

T. (ºC)	Days	Treat.	H_2O_2 Leaves	H_2O_2 Roots	Proline Leaves	Proline Roots
			(μmol g^{-1} FW)		(μg g^{-1} DW)	
25	0	+Mg	13 Aa	2.9 Aa	1.1 Aa	0.4 Aa
	10		17 Ab	2.8 Aa	1.0 Bb	0.5 Ab
	20		16 Bb	2.8 Bb	1.2 Bb	0.6 Bb
	30		16 Bb	3.1 Bb	1.2 Bb	0.7 Bb
	0	-Mg	13 Aa	2.9 Aa	1.1 Aa	0.4 Aa
	10		17 Ab	3.5 Aa	1.5 Ab	0.6 Ab
	20		22 Ab	4.2 Ab	1.7 Ab	0.9Ab
	30		31 Ab	4.6 Ab	1.6 Ab	0.9 Ab
35	0	+Mg	13 Aa	2.9 Aa	1.1 Aa	0.4 Aa
	10		19 Ba	3.2 Ba	4.3 Ba	1.2 Ba
	20		24 Ba	3.9 Ba	3.5 Ba	1.4 Ba
	30		29 Ba	3.9 Ba	4.9 Ba	3.4 Ba
	0	-Mg	13 Aa	2.9 Aa	1.1 Aa	0.4 Aa
	10		30 Aa	4.1 Aa	5.5 Aa	1.4 Aa
	20		32 Aa	5.2 Aa	4.7 Aa	2.2 Aa
	30		37 Aa	5.8 Aa	6.6 Aa	4.1 Aa

T.: temperature; Treat.: treatment; DW: dry weight; FW: fresh weight.

Mg-deficiency and temperature rise from 25 to 35 ° C resulted in increases in superoxide dismutase (SOD) activity (Figure 1). On the thirtieth day, Mg deficiency triggered an increase in SOD activity of 22% in leaves and 13% in roots. Heat stress, in turn, increased the activity of this enzyme in 66% for leaves and in 171% for roots. The combination of Mg deficiency and temperature rise resulted in higher superoxide dismutase activity, even higher than observed in each one alone.

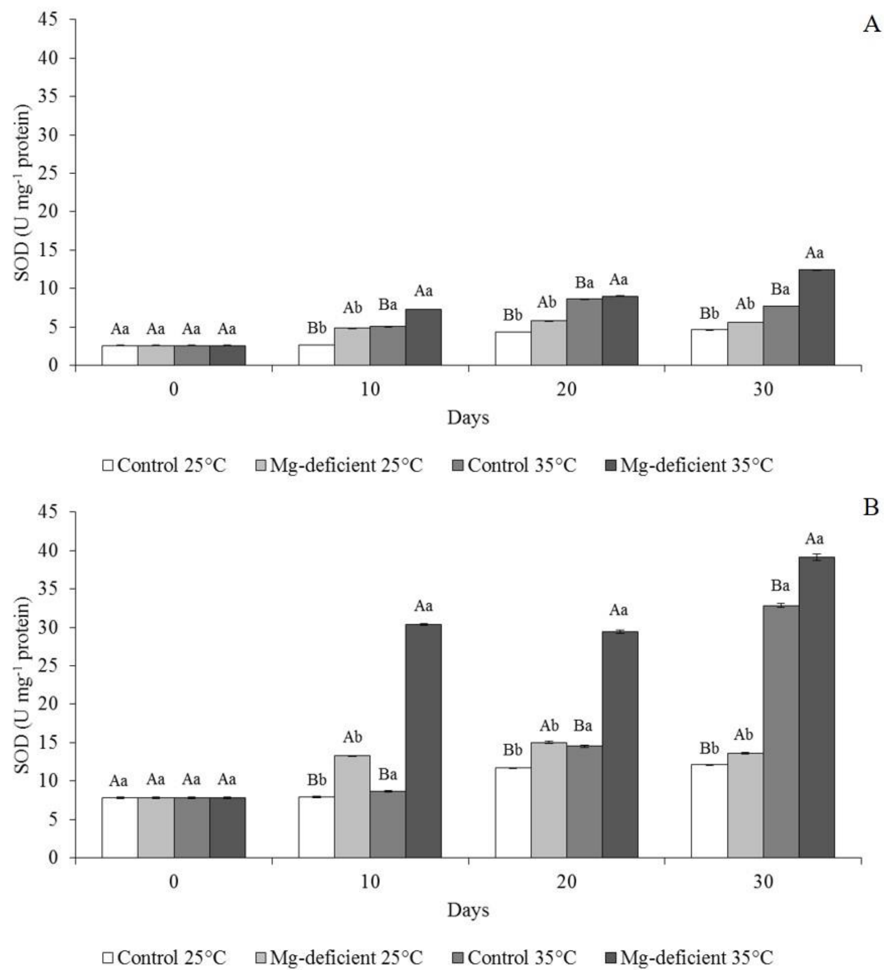

Figure 1: Superoxide dismutase (SOD) activity in leaves (A) and roots (B) of *Coffea arabica* L. seedlings submitted to different concentrations of magnesium and temperatures. Capital letters compare the effect of magnesium deficiency on each temperature and sampling time and lowercase letters compare the effect of temperature elevation on each Mg condition and sampling time. Different letters indicate significant differences with 0.05 probability, according Scott-Knott test. Bars show the standard error of five replicates

Catalase (CAT) activity was elevated by Mg deficiency and by heat stress (Figure 2). At the end of the experimental time, leaves and roots of seedlings without Mg showed increases in CAT activity of 119 and 109%, respectively. In addition, temperature rise triggered an increase of 21 and 149% in the catalase activity in leaves and roots of coffee, respectively. When we submitted *Coffea arabica* L. seedlings to both stresses, CAT activity reached higher values than those found in each stress separately, both in leaves and roots.

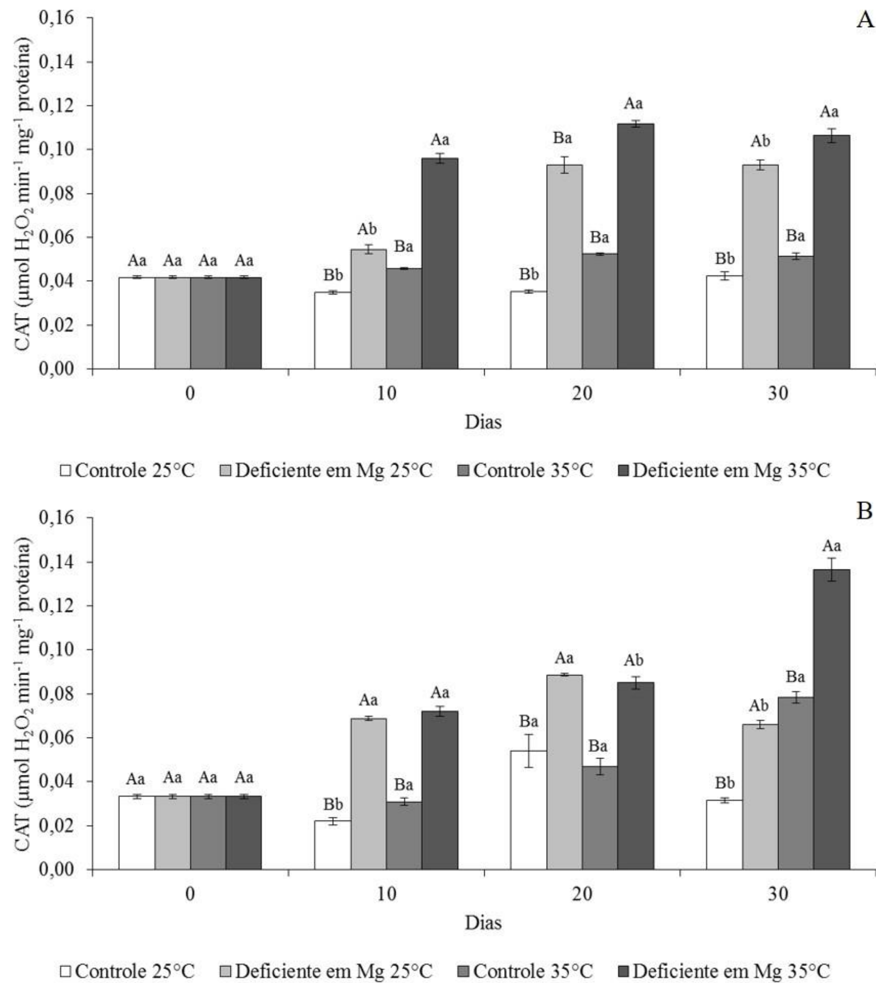

Figure 2: Catalase (CAT) activity in leaves (A) and roots (B) of *Coffea arabica* L. seedlings submitted to different concentrations of magnesium and temperatures. Capital letters compare the effect of magnesium deficiency on each temperature and sampling time and lowercase letters compare the effect of temperature elevation on each Mg condition and sampling time. Different letters indicate significant differences with 0.05 probability, according Scott-Knott test. Bars show the standard error of five replicates

Coffee seedlings submitted to combination of stresses (-Mg/35ºC) showed the highest activities of ascorbate peroxidase (APX; Figure 3), higher than those observed in seedlings exposed to each of these stresses alone. At the end of the experimental period, Mg deficiency resulted in increases in APX activity of 79 and 40% in leaves and roots, respectively. While heat stress resulted in an increase in APX activity of 38%, but only in leaves.

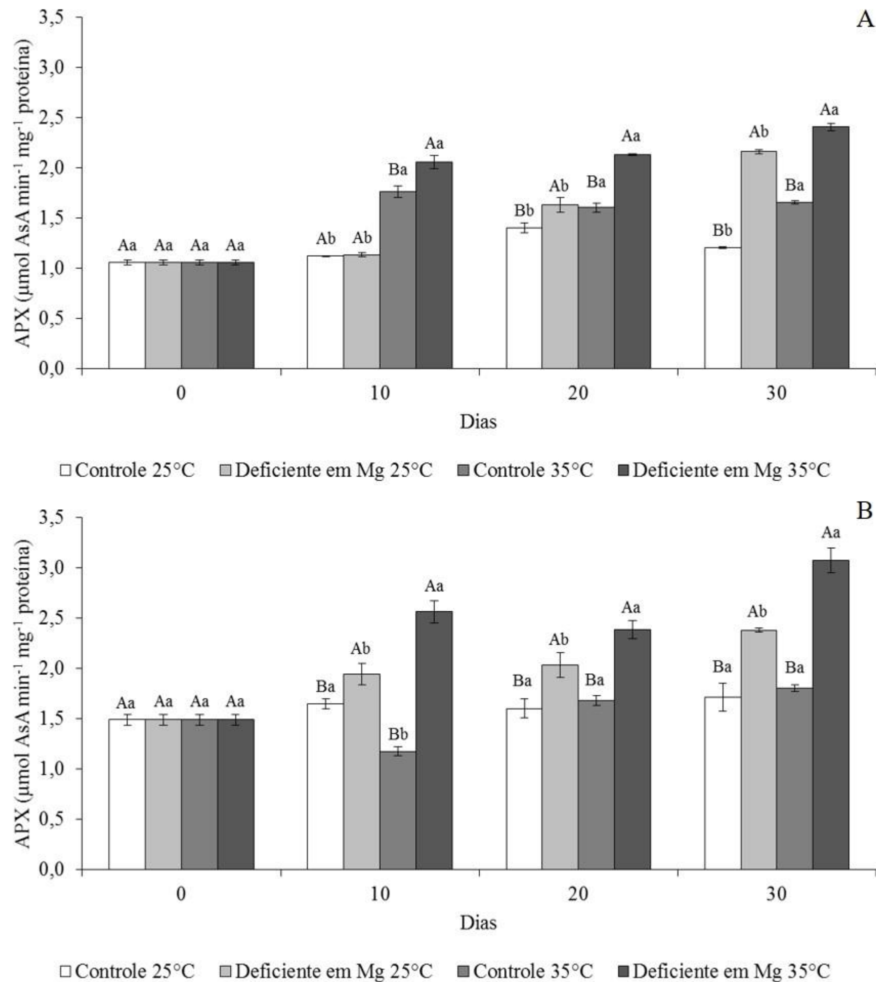

Figure 3: Ascorbate peroxidase (APX) activity in leaves (A) and roots (B) of *Coffea arabica* L. seedlings submitted to different concentrations of magnesium and temperatures. Capital letters compare the effect of magnesium deficiency on each temperature and sampling time and lowercase letters compare the effect of temperature elevation on each Mg condition and sampling time. Different letters indicate significant differences with 0.05 probability according Scott-Knott test. Bars show the standard error of five replicates

Leaves and roots of Mg-deficient seedlings had higher ascorbate concentration than those of control, for both temperatures (Figure 4). However, this increase was observed from

tenth day for seedlings grown at 35 °C and from 20th day for seedlings grown at 25 °C. Thus, on the 30th day, leaves and roots of seedlings submitted to magnesium deficiency and grown at 25 °C presented 11% more ascorbate than those of the control. Temperature rise, alone, unaffected ascorbate concentration, while combinatory effect of Mg-deficiency and heat stress culminated in an increment of 31 % on ascorbate levels in leaves and roots.

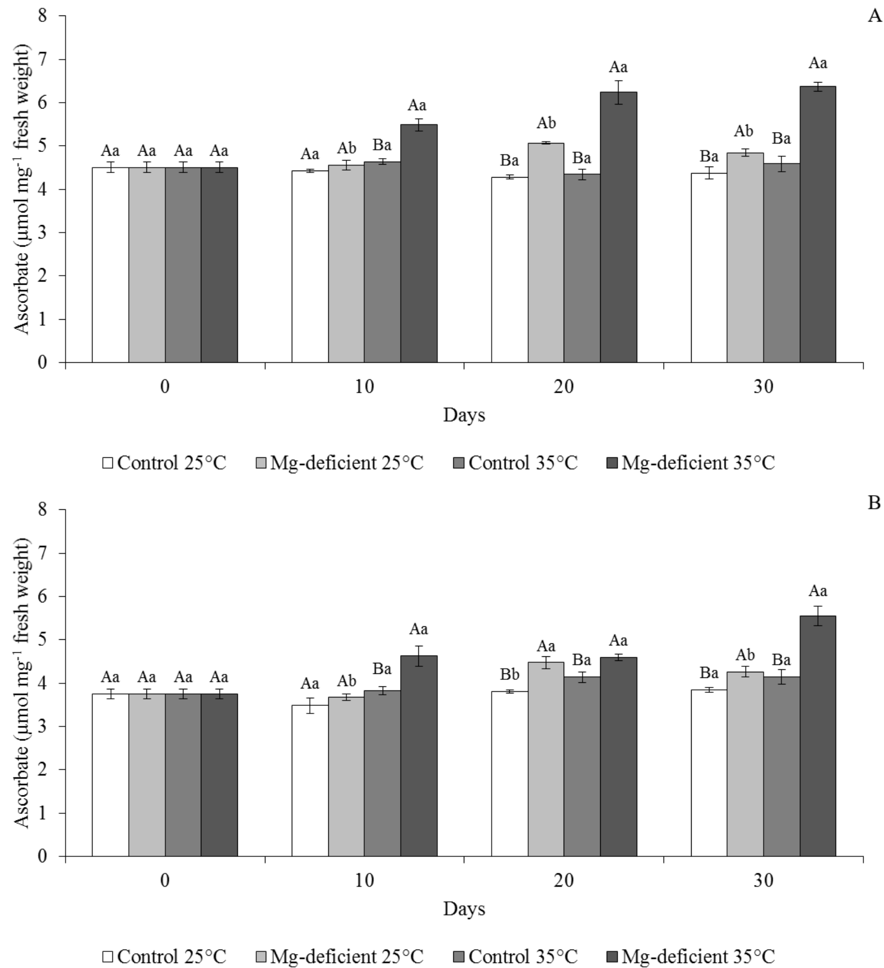

Figure 4: Ascorbate concentration in leaves (A) and roots (B) of *Coffea arabica* L. seedlings submitted to different concentrations of magnesium and temperatures. Capital letters compare the effect of magnesium deficiency on each temperature and sampling time and lowercase letters compare the effect of temperature elevation on each Mg condition and sampling time. Different letters indicate significant differences with 0.05 probability according Scott-Knott test. Bars show the standard error of five replicates

Mg-deficiency and temperature rise from 25 to 35 ° C resulted in increases in monodehydroascorbate reductase (MDHAR) activity (Table 2). On the thirtieth day, Mg

deficiency triggered an increase in MDHAR activity of 73% in leaves and 31% in roots. Heat stress, in turn, increased in 55 and 52% the activity of this enzyme in leaves and roots, respectively. Combination of Mg deficiency and temperature rise resulted in higher values of MDHAR activity, even higher than observed in each stress alone.

At the end of the experimental period, combination of Mg deficiency and heat stress resulted in higher activity of the dehydroascorbate reductase (DHAR) in leaves and roots (Table 2). This elevation was superior even to those observed in seedlings submitted to each of these stresses alone. Seedlings of *Coffea arabica* L. submitted to Mg deficiency showed an increase in DHAR activity of 173% in leaves and 44% in roots. The exposure of seedlings to heat stress, in turn, led to an increase in activity of this enzyme of 380% in leaves and 92% in roots at the end of the experimental period.

Combination of Mg deficiency and heat stress resulted in the highest values of glutathione reductase (GR) activity at day 30 (Table 2). However, even in isolation both Mg exclusion and heat triggered elevations in GR activity. Leaves and roots of seedlings without Mg presented in the last evaluation an increase in GR activity of 117 and 52%, respectively. While, temperature rise led to an increase in glutathione reductase activity, at day 30, of 147% in leaves and 222% in roots.

Table 2: Monodehydroascorbate reductase, dehydroascorbate reductase and glutathione reductase in leaves and roots and roots of *Coffea arabica* L. seedlings submitted to different concentrations of magnesium and temperatures. Capital letters compare the effect of magnesium deficiency on each temperature and sampling time and lowercase letters compare the effect of temperature elevation on each Mg condition and sampling time. Different letters indicate significant differences with 0.05 probability according Scott-Knott test.

T. (ºC)	Days	Treat.	MDHAR leaves (µmol ascorbate min^{-1} mg^{-1} protein)	MDHAR roots	DHAR leaves	DHAR roots	GR leaves (µmol NADPH min^{-1} mg^{-1} protein)	GR roots
25	0	+ Mg	18 Aa	67 Aa	0,1 Aa	0,2 Aa	0,07 Aa	0,12 Aa
	10		26 Bb	62 Bb	0,0 Bb	0,1 Bb	0,05 Bb	0,15 Bb
	20		21 Bb	79 Bb	0,1 Bb	0,1 Bb	0,06 Bb	0,14 Bb
	30		29 Bb	76 Bb	0,1 Bb	0,2 Bb	0,04 Bb	0,11 Bb
	0	- Mg	18 Aa	67 Aa	0,1 Aa	0,2 Aa	0,07 Aa	0,12 Aa
	10		40 Ab	94 Ab	0,1 Ab	0,2 Ab	0,10 Aa	0,29 Aa
	20		49 Aa	93 Ab	0,1 Ab	0,2 Ab	0,08 Ab	0,26 Ab
	30		51 Ab	100 Ab	0,2 Ab	0,3 Ab	0,09 Ab	0,17 Ab
35	0	+ Mg	18 Aa	67 Aa	0,1 Aa	0,2 Aa	0,07 Aa	0,12 Aa
	10		49 Aa	148 Ba	0,1 Aa	0,3 Ba	0,09 Aa	0,19 Ba
	20		40 Ba	150 Ba	0,2 Ba	0,4 Ba	0,11 Ba	0,23 Ba
	30		45 Ba	116 Ba	0,3 Ba	0,4 Ba	0,11 Ba	0,36 Ba
	0	- Mg	18 Aa	66,7 Aa	0,1 Aa	0,2 Aa	0,07 Aa	0,12 Aa
	10		50 Aa	172,2 Aa	0,1 Aa	0,3 Aa	0,09 Aa	0,27 Aa
	20		48 Aa	174 Aa	0,3 Aa	0,4 Aa	0,17 Aa	0,41 Aa
	30		60 Aa	179 Aa	0,4 Aa	0,6 Aa	0,17 Aa	0,41 Aa

T.: temperature; Treat.: treatment.

The levels of malondialdehyde (Figure 5), a byproduct of lipid peroxidation, reached their highest values due to combination of stresses (-Mg/35 ºC) since the second evaluation. At day 30, leaves and roots of seedlings without Mg at 25 ºC showed an increase in malondialdehyde production of 41 and 38%, respectively. Elevation of temperature from 25 to 35 ° C, alone, resulted in an increase in lipid peroxidation of 45% in both leaves and roots.

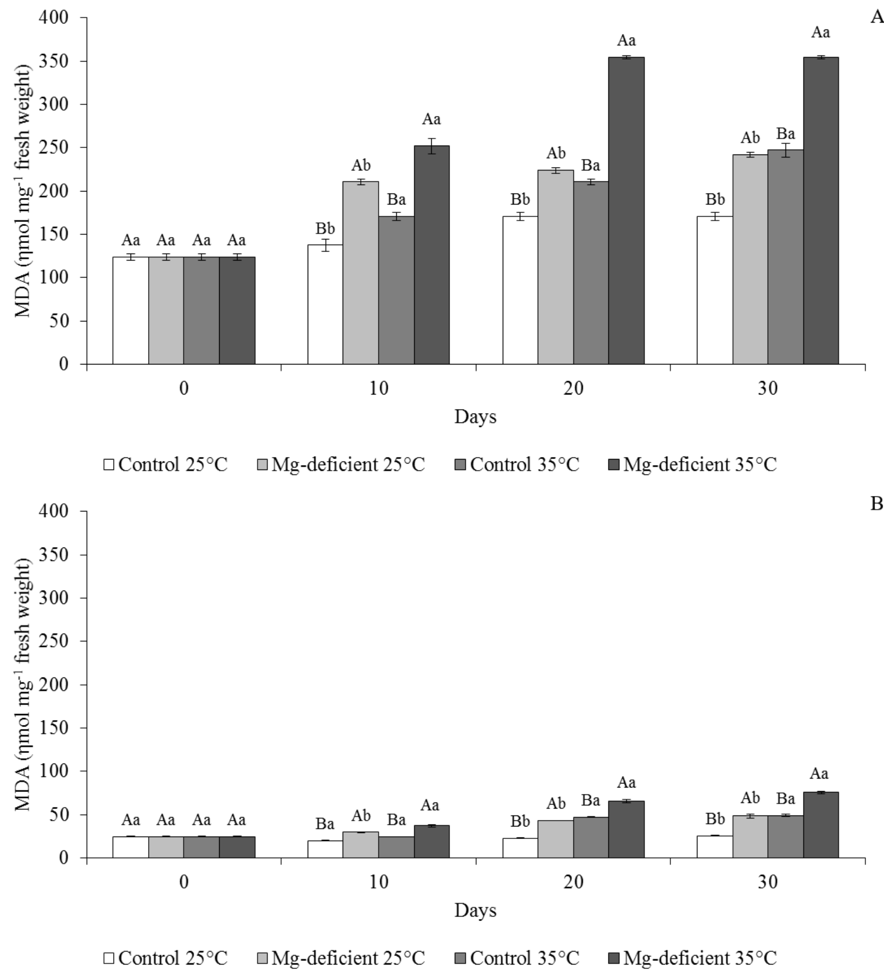

Figure 5: Malondialdehyde concentration in leaves (A) and roots (B) of *Coffea arabica* L. seedlings submitted to different concentrations of magnesium and temperatures. Capital letters compare the effect of magnesium deficiency on each temperature and sampling time and lowercase letters compare the effect of temperature elevation on each Mg condition and sampling time. Different letters indicate significant differences with 0.05 probability according Scott-Knott test. Bars show the standard error of five replicates

Combination of magnesium deficiency and heat stress resulted in lower levels of protein (Figure 6), including those found in each of these stresses alone. In leaves, Mg deficiency and heat stress triggered reductions of 31% and 10%, respectively, in protein concentration. In roots, this reduction was of 33% due to the Mg deficiency and 13% due to the elevation of temperature from 25 to 35 ºC.

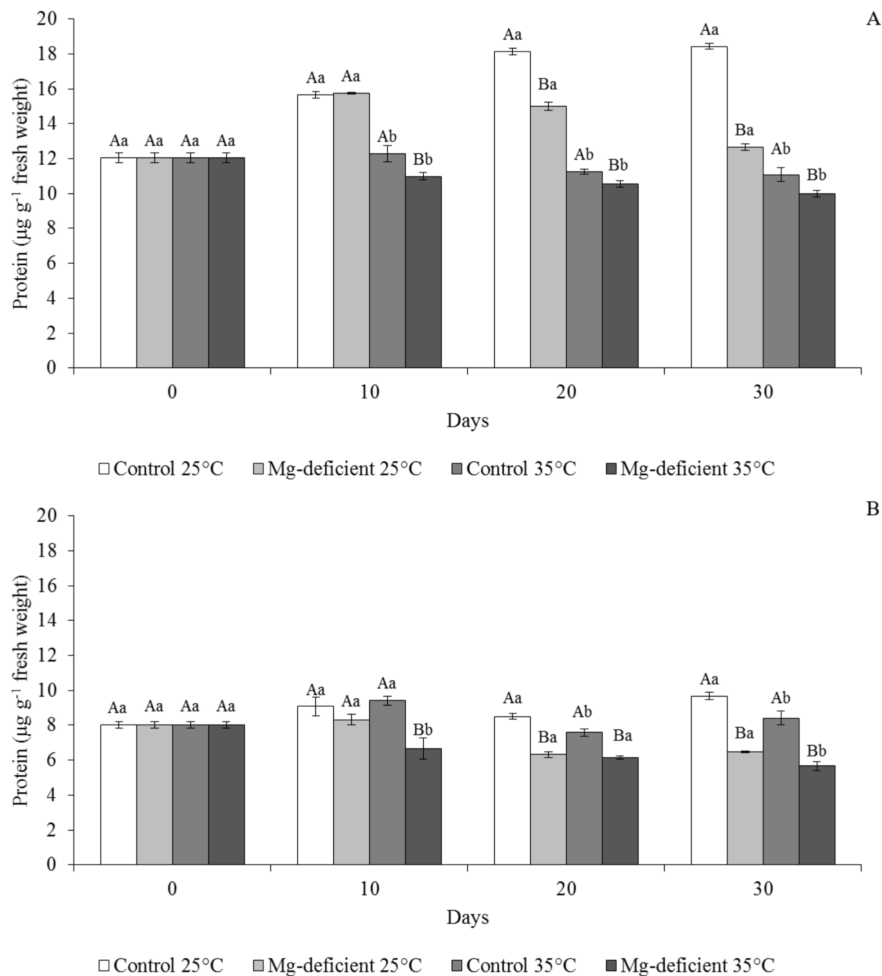

Figure 6: Protein concentration in leaves (A) and roots (B) of *Coffea arabica* L. seedlings submitted to different concentrations of magnesium and temperatures. Capital letters compare the effect of magnesium deficiency on each temperature and sampling time and lowercase letters compare the effect of temperature elevation on each Mg condition and sampling time. Different letters indicate significant differences with 0.05 probability according Scott-Knott test. Bars show the standard error of five replicates

Seedlings of *Coffea arabica* L. without Mg showed increase in amino acid levels (Figure 7). At day 30, this increment was of 19% for leaves and 26% for roots. Heat stress, alone, was responsible for increases in amino acids concentrations of 43 for leaves and 57% for roots. Combination of Mg deficiency and heat stress resulted in the highest concentration of amino acids, at the end of experimental period.

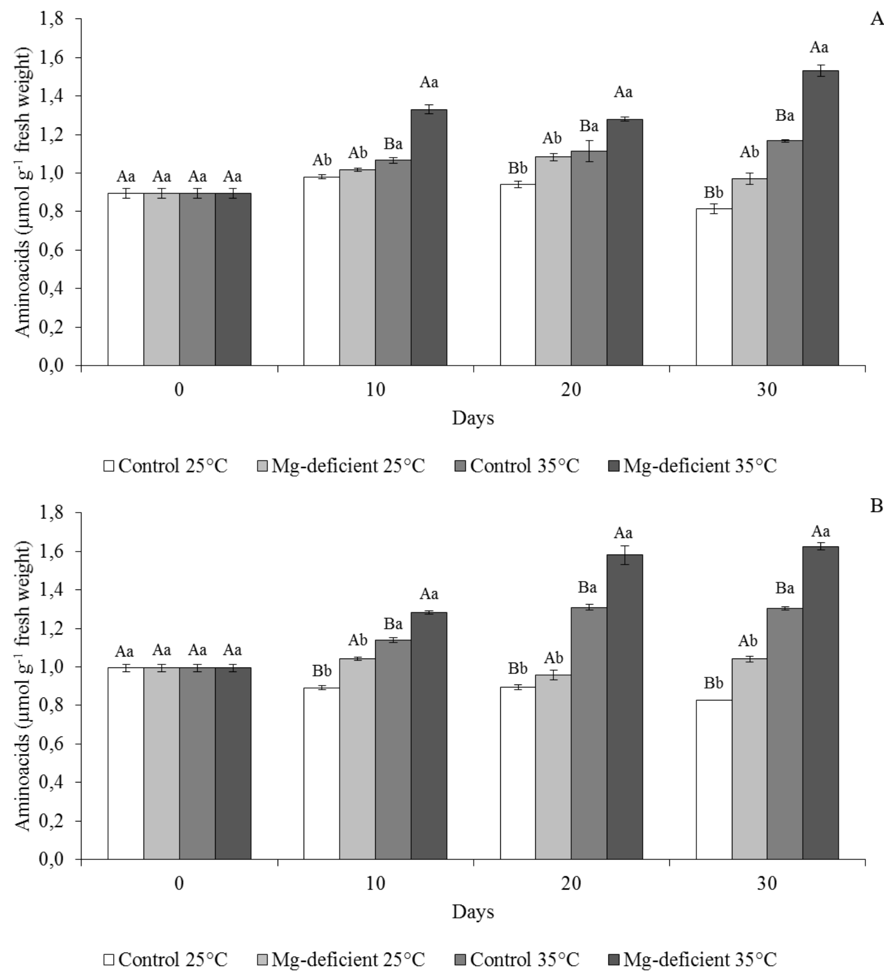

Figure 7: Amino acid concentration in leaves (A) and roots (B) of *Coffea arabica* L. seedlings submitted to different concentrations of magnesium and temperatures. Capital letters compare the effect of magnesium deficiency on each temperature and sampling time and lowercase letters compare the effect of temperature elevation on each Mg condition and sampling time. Different letters indicate significant differences with 0.05 probability according Scott-Knott test. Bars show the standard error of five replicates

Discussion

Mg deficiency and heat stress have the alteration in carbohydrate partitioning as one of their main effects. The increase in leaf concentration of photoassimilates acts as a negative feedback mechanism that inhibit enzymes from the biochemical step of photosynthesis (Hermans et al. 2004). The interruption of CO_2 fixation, in turn, can induce the saturation of electron transport chain due to the accumulation of NADPH (Hermans and Verbrudgen 2005; Silva et al. 2014). High levels of reducing equivalents and components of the electron transport

chain saturated create favorable conditions for reactive oxygen species (ROS) generation (Mittler 2002). In the present work, was observed an increase in H_2O_2 production due to both stress, mainly by stress combination (Table 1).

A key adaptive mechanism developed by many plant species when subjected to abiotic stresses is the accumulation of certain organic compounds, generally referred to as compatible osmolytes (Asthir 2015). Proline, glycine betaine and soluble sugars are some of these osmoprotectants and are involved in the protection and stabilization of proteins and membranes against the oxidative action of reactive oxygen species (Kumar et al. 2012). Although increases in proline production under Mg deficiency have not previously been reported, our results (Table 1) pointed to the need of future studies, considering the different roles played by proline in plant metabolism under stress conditions.

Proline accumulation is considered an important adaptive mechanism of plants subjected to temperature elevation, since is the first metabolite to participate directly in osmotic adjustment (Sakamoto and Murata 2000). Moreover, the increase in proline, glycine betaine and soluble sugars is fundamental in the protection of cellular structures against damages caused by heat (Farooq et al. 2008). The increase in proline production can be also associated with elevation in xanthophyll biosynthesis, which are pigments responsible for protection against oxidative damage triggered by heat (Dobra et al. 2010). In coffee leaves, proline levels were correlated to H_2O_2 production (0.74), showing that the increase in proline production is a response to elevation in ROS levels.

Since NADPH is essential in proline biosynthesis process, Hare and Cress (1997) have observed that even a small increase in proline biosynthesis has a large impact on the cellular pool of this reducing agent. Thus, increases in proline production (Table 1) under Mg deficiency and heat stress may also play an important role in avoiding ROS formation by consuming the excess of reducing power. In addition, Hare and Cress (1997) suggest that proline degradation after the stress period may be responsible for providing reducing equivalents to support the generation of ATP mitochondrial required for post-stress recovery.

However, in despite of high proline production at day 30, high levels of H_2O_2 were still observed (Table 1). This hydrogen peroxide can come from both photorespiration and dismutation of superoxide radical by the action of superoxide dismutase (SOD). SOD action is inherent to all subcellular compartments and provides the first line of defense against the toxic effects of high ROS levels (Gill and Tuteja 2010). In addition, removing O_2^- SOD reduces the risk of hydroxyl radical production through Haber-Weiss reactions (Sharma et al. 2012).

Mg deficiency and heat stress were responsible for raising activity of the superoxide dismutase (Figure 1). However, on the thirtieth day, the increase in SOD activity due to temperature rise was higher than that triggered by Mg exclusion. The superoxide dismutase activity in leaf tissue showed a correlation of 0.83 with the hydrogen peroxide levels, showing that the increase in the SOD activity is related to higher H_2O_2production, which must then be removed by the action of the enzymes catalase and ascorbate peroxidase.

Catalase (CAT) is one of the enzymes involved in H_2O_2 removal process and is localized in the peroxisomes, cytosol and mitochondria. However, among the enzymes involved in this process, CAT has low affinity for H_2O_2, acting preferable under high H_2O_2 concentrations (Gill and Tuteja 2010). In the present study, leaves of coffee seedlings submitted to both stress showed high hydrogen peroxide concentrations. Perhaps, this high H_2O_2 levels could be responsible for correlation of 0.73 between H_2O_2 concentration and catalase activity in leaves. In addition, APX was also involved in H_2O_2 removal in leaves with a correlation of 0.87. On the other hand, for roots it was observed lower H_2O_2 concentrations and a non-significant correlation between levels of this reactive oxygen species and catalase activity. However, a correlation of 0.63 between H_2O_2 concentration and APX activity was observed in roots, which corroborates with the fact that this enzyme have greater affinity for peroxide, acting under low H_2O_2 concentration are observed (Sharma et al. 2012).

Lower activity of CAT in plants of *Citrus reticulata* subjected to Mg deficiency may be a reflection of a lower rate of photorespiration, since a reduction in Rubisco activity was observed (Tang, Lu and Chen 2012). As a result of the reduction in photorespiration, there would be less H_2O_2production, and under such conditions, the most efficient enzyme in the ROS removal would be ascorbate peroxidase, which has a higher affinity for hydrogen peroxide (Gill and Tuteja 2010). Higher CAT activity in *Coffea arabica* L. seedlings submitted to heat stress could be explained by the increase in photorespiration rates, since H_2O_2 production by this process is responsible for most of H_2O_2 formed in C3 species (Noctor et al. 2002).

Higher SOD, CAT and APX activity has been considered a suitable biochemical marker for the determination of critical mineral concentrations and can be considered as one of the first physiological responses to mineral stress in plants (Blasco; Grahan; Broadley 2015). Combination of Mg deficiency and heat stress in *Coffea arabica* L. seedlings resulted in higher SOD, CAT and APX activities (Figures 1, 2 and 3) as well as higher proline levels (Table 1), showing that antioxidant metabolism is active in order to mitigate the negative effects of high ROS production. However, H_2O_2 concentration at the thirtieth day remained high (Table 1),

showing that despite antioxidant system response, its action was not enough to remove excess reactive oxygen species produced.

APX acts converting H_2O_2 into H_2O while oxidizes ascorbate to monodehydroascorbate (MDHA). MDHA, in turn, needs to be further reduced in order to maintain ascorbate levels for ascorbate peroxidase activity. The reduction of monodehydroascorbate to ascorbate can be accomplished by monodehydroascorbate reductase (MDHAR) through the water-water cycle, or by the action of the dehydroascorbate reductase (DHAR) and glutathione reductase (GR) through the ascorbate-glutathione cycle. In water-water cycle, MDHAR reduces MDHA to ascorbate using NADH preferentially to NADPH as an electron donor. In the ascorbate-glutathione cycle, MDHA is first converted to dehydroascorbate (DHA) by non-enzymatic reactions. DHA is then reduced to ascorbate by the action of DHAR, which uses glutathione as an electron donor. Then, oxidized glutathione must be reduced to maintain the activity of dehydroascorbate reductase. This reduction is performed by glutathione reductase and has NADPH as an electron donor (Sharma et al. 2012).

Glutathione reductase is a potential enzyme in the ascorbate-glutathione cycle and plays an essential role in the antioxidant defense system by keeping the pool of glutathione reduced, since reduced glutathione is essential in the regeneration of dehydroascorbate by the enzyme dehydroascorbate reductase (Gill and Tuteja 2010). In the process of reducing glutathione, GR consumes the excess of reducing power. Thus, in addition to being part of antioxidant metabolism, GR can reduce the favorable conditions for ROS production by consuming reducing power in excess.

Ascorbate levels in leaves of *Coffea arabica* L. correlated with APX (0.63), MDHAR (0.61) and GR (0.68) activity. Roots, in turn, showed a correlation of 0.73, 0.71, 0.76 and 0.64 between ascorbate levels and the activity of the APX, MDHAR, DHAR and GR. As the pool of ascorbate available for the action of ascorbate peroxidase depends on the action of the enzymes MDHAR, DHAR and GR, it is more than expected that the concentration of this antioxidant molecule and the activity of these enzymes exhibit similar behaviors. This joint action of antioxidant molecules and enzymes is fundamental in removal of reactive oxygen species. However, when ROS production exceeds the removal capacity of antioxidant metabolism, this system is not able to provide sufficient protection to membrane lipids, resulting in a significant increase in malondialdehyde levels, a byproduct of lipid peroxidation (Farhat et al. 2016).

Membrane peroxidation compromises the functioning of ion channels that are inserted in lipid bilayer and that ensure its selectivity. Therefore, malfunction of ionic channels has as

main consequence the impairment of permeability through membrane (Asthir 2015). Thus, the main effect of lipid peroxidation is the damage to membrane permeability. In addition, it is necessary to emphasize that membranes have in their structure heat-sensitive sensors, which help the plants in the activation of its defense mechanisms (Kumar et al. 2012). Therefore, the impairment of these receptors by lipid bilayer peroxidation may alter the response of the plant to stress. Thus, the stability of the membrane can be considered an important parameter of tolerance of plants to several stresses, mainly to heat stress.

Reduction in protein concentration (Figure 6) and the consequent increase in amino acid levels (Figure 7) due to Mg deficiency can be explained by the function of magnesium in the protein synthesis process. Ribosomes are macromolecular structures responsible for protein biosynthesis and their active form requires the aggregation of two subunits and Mg form a bridge between them (Maathuis 2009). Thus, protein biosynthesis is strongly reduced under Mg deficiency leading to an increase in amino acids concentrations, their precursors (Fischer et al. 1998). In roots of coffee seedlings, a correlation of 0.78 between Mg and protein levels was observed, corroborating with the hypothesis that the reduction in Mg concentration is responsible for the reduction in protein biosynthesis and the consequent increase in amino acids levels.

On the other hand, heat stress has as one of main effects the inhibition of protein synthesis as well as its denaturation (Howarth 2005). It is believed that the degradation of proteins due to heat can be accelerated by the stimulation in the synthesis of proteolytic enzymes, which catalyze the hydrolysis of peptide bonds (Cooke; Roberts; Davies 1980). In addition, the decrease in protein content may be related to the increase in the production of reactive oxygen species (Gill and Tuteja 2010). Coffee leaves had a correlation of -0.83 between hydrogen peroxide levels and proteins concentration, suggesting the performance of ROS in protein denaturation.

Heat stress is a growing concern in production of many crops due to the current global warming. Mg deficiency, in turn, has become an important nutritional deficiency in agricultural soils. Particularly in *Coffea arabica* L., since coffee crops has been implemented at agricultural frontiers, where soils are acid, with low cation exchange capacity and poor in magnesium. In *Coffea arabica* L., the effects of temperature rise on antioxidant metabolism are more pronounced when seedlings are simultaneously exposed to Mg deficiency. When submitted to heat stress, coffee seedlings under adequate Mg nutrition, showed lower production of hydrogen peroxide and consequently poor lipid peroxidation and protein denaturation than those deficient

in Mg. Therefore, an adequate Mg nutrition is essential to minimize oxidative damage caused by heat stress in coffee seedlings.

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