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Dissertação

Nutrição e aporte hídrico: alterações bioquímico-fisiológicas e moleculares em morangos cv. Camarosa

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Nutrição e aporte hídrico: alterações bioquímico-fisiológicas e moleculares em morangos cv. Camarosa

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“Não basta a leitura sem a unção, a especulação sem a devoção, a pesquisa sem maravilhar-se; a circunspecção sem o júbilo, o trabalho sem a piedade, a ciência sem a caridade, a inteligência sem a humildade, o estudo sem a graça”.

(SÃO BOAVENTURA)

Resumo

PERIN, Ellen Cristina. **Nutrição e aporte hídrico: alterações bioquímico-fisiológicas e moleculares em morangos cv. Camarosa.** 2014. 117f. Dissertação (Mestrado). Programa de Pós-graduação em Ciência e Tecnologia de Alimentos. Universidade Federal de Pelotas, Pelotas - RS.

A biofortificação de culturas agrícolas é uma estratégia que vem sendo estudada e explorada com o objetivo de complementar da alimentação pelo incremento ou melhoria na qualidade dos alimentos, visando reduzir a desnutrição e oferecer alimentos mais saudáveis. Dentre as técnicas utilizadas para realizar a biofortificação, o uso de fertilização e, mais recentemente, a aplicação de estresses abióticos controlados vem sendo abordadas, mostrando que as aplicações dessas técnicas podem acarretar no acúmulo de compostos com potencial antioxidante de interesse para promoção da saúde humana, sem prejudicar significativamente o desenvolvimento e produção das culturas. Os frutos de morango, cujas características sensoriais específicas vem promovendo aumento do seu consumo, apresentam naturalmente em sua composição compostos com potencial antioxidante, como vitamina C, compostos fenólicos e antocianinas os quais podem ter seus teores modificados de forma a melhorar seu potencial antioxidante, além de suas funções em condições de estresse como defesa. Por outro lado, o cultivo do morangueiro apresenta uma alta taxa de sensibilidade a déficits hídricos e nutricionais; a adubação empregada nessa cultura normalmente utiliza fontes solúveis de nutrientes minerais, que podem acarretar em prejuízos ao solo, desequilíbrio nutricional, lixiviação e contaminação de lençóis freáticos. Neste estudo, plantas de morangos foram submetidas a diferentes níveis de estresse hídrico e a uma adubação cuja matriz foi composta de torta de tungue e pós-de-rocha, efetuando-se avaliações de variáveis bioquímico-fisiológicas e moleculares. Os resultados mostraram que a adubação alternativa apresentou potencial para utilização como insumo agrícola, uma vez que aumentou a produção de frutos (23,79 % a mais que a adubação solúvel) tendo ainda proporcionado um maior acúmulo de compostos com potencial antioxidante nos frutos em comparação à adubação solúvel convencional (antocianinas, acúmulo relativo do transcrito *UFGT* e ácido L-ascórbico). A avaliação desta adubação alternativa em diferentes níveis de estresse hídrico demonstrou haver um maior incremento nos compostos fenólicos e atividade antioxidante, evidenciando a viabilidade desta estratégia de biofortificação, sendo que o nível 1 de estresse apresentou menores perdas em relação aos parâmetros fotossintéticos avaliados e redução da produção de frutos em relação ao nível de estresse mais severo avaliado (nível 2). Os resultados obtidos neste estudo corroboram o potencial de uso da adubação alternativa avaliada no cultivo do morangueiro, sendo necessária ainda a busca por um nível de estresse hídrico que além de incrementar compostos com potencial antioxidante, conforme observado neste estudo, não afete significativamente o desenvolvimento das plantas e a produtividade da cultura do morangueiro, bem como sirva como uma técnica eficaz de biofortificação.

Palavras-chave: biofortificação; estresse hídrico; adubação alternativa; *Fragaria x ananassa*; produção; qualidade.

Abstract

PERIN, Ellen Cristina. **Nutrition and fluid intake: Biochemical-physiological and molecular changes in strawberry cv. Camarosa.** 2014. 117f. Dissertation (Master Degree in Food Science and Technology) – Federal University of Pelotas, Pelotas – RS.

The biofortification of crops is a strategy that has been studied and explored in order to improve feeding supply by increasing or improving food quality of food to reduce malnutrition and offer healthier foods. Among the techniques used to perform the biofortification, the use of fertilization and, more recently, the application of controlled abiotic stresses have been applied, showing that the application of these techniques may result in the accumulation of antioxidant compounds with potential interest for promotion of human health, without significantly harming crop development and productivity. The strawberry fruits, whose specific sensorial features make its increasingly high consumption, naturally present in its composition, with compounds with potential antioxidant such as vitamin C, phenolic acids and anthocyanins, which may have their contents modified in order to improve their antioxidant potential in addition to its defense functions under stress conditions. On the other hand, the strawberry crop has a high rate of sensitivity to water and nutritional deficits; main fertilizer strategies for this crop typically uses soluble sources of nutrients, which can result in soil damage, nutritional imbalance, leaching and contamination of groundwater. In this study, strawberry plants were subjected to different levels of water stress and to an alternative fertilizer matrix composed of tung presscake and rock powder, and evaluated to biochemical, physiological and molecular variables. The results showed that the alternative fertilization has potential for use as an agricultural input as long as it has increased the fruit yield (23.79 % related to the soluble fertilizer treatment) and also improved the accumulation of L-ascorbic acid, anthocyanins and the related expression of the *UFGT* gene compared to the soluble fertilizer strategy. The evaluation of the same alternative fertilizer at two different levels of water stress showed a significant increase in total phenolic compounds and antioxidant activity, demonstrating the feasibility of this biofortification strategy. In the level 1 of drought stress the results showed an increase in the photosynthetic parameters and in the fruit productivity related to the more severe level of stress (level 2). The results of this study support the potential use of the alternative fertilization evaluated for the strawberry crop, although that remains necessary studies to find out drought stress level that besides increasing compounds with antioxidant potential, as observed in this study, do not affect significantly the plant growth and yield of strawberry crop, to be used as an biofortification technique.

Key words: biofortification; drought stress; alternative fertilizer; *Fragaria x ananassa*; yield; fruit quality.

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Sumário

1. Introdução

Estratégias vêm sendo desenvolvidas visando atender à necessidade de diminuição das discrepâncias alimentares entre continentes, regiões e povos, para prover reservas frente ao crescimento populacional, bem como buscar alternativas para produzir alimentos com valores nutricionais e funcionais incrementados e também pelo aumento da procura por parte dos consumidores por alimentos mais saudáveis. Essas medidas também podem promover a complementação da alimentação, por meio do incremento ou melhora na qualidade dos alimentos, na tentativa de combater a desnutrição e atender à procura por alimentos mais saudáveis. Dentre essas estratégias destacam-se aquelas políticas, com ênfase à segurança alimentar, ou seja, produzir alimentos em quantidade e com qualidade, num contexto em que se busque a equidade de acesso ao consumo.

No plano tecnológico e científico, a biofortificação de culturas agrícolas é uma das estratégias através da qual é possível complementar as intervenções existentes para fornecer micronutrientes e moléculas bioativas com uma boa relação custo-benefício, melhorando o potencial nutricional e funcional dos alimentos (SALTZMAN et al., 2013; MESSIAS et al., 2013). A aplicação de estresses abióticos moderados consiste em um dos meios de biofortificação de culturas agrícolas (PALMGREN et al., 2008).

A cadeia produtiva de morangos no Brasil desempenha um importante papel sócio-econômico (EMBRAPA, 2011). No Estado do Rio Grande do Sul, por exemplo, a maior produção se concentra nos municípios do Vale do Rio Caí, Serra Gaúcha e na região de Pelotas (MENDONÇA, 2011). O morango, em sua composição química, possui compostos antioxidantes naturais, vitaminas, minerais, compostos fenólicos, dentre outros como alcalóides, ácidos orgânicos, etc (ERKAN et al., 2008). Sob o ponto de vista agronômico, trata-se de uma cultura sensível ao aporte nutricional e hídrico (LIU et al., 2007; BAMBERG, 2010).

Uma das práticas mais importantes no plantio do morangueiro é a adubação, pela sua influência na produtividade, conservação pós-colheita e qualidade dos frutos (PREZOTTI, 2006). Nos últimos anos, a preocupação com a segurança alimentar e prevenção da poluição ambiental tem estimulado a busca por adubações que melhorem essas características (PESAKOVIC et al., 2013). A adequação de práticas agronômicas às exigências dos consumidores é o meio para produção de

alimentos mais saudáveis, nutritivos e ecologicamente corretos, sendo, no entanto um desafio para o produtor e para a pesquisa (DAROLT, 2008). Muitos estudos vêm analisando o potencial da adubação organomineral pois ela apresenta diversos outros nutrientes, além dos nutrientes básicos (nitrogênio, fósforo e potássio) (MELO JÚNIOR et al., 2012) e visa a obtenção de alimentos com potencial nutricional e funcional e diminuição de custos, além de benefícios ambientais e aproveitamento de resíduos, pela utilização, por exemplo, de pós de rocha (obtidos pela prática de mineração) e tortas de oleaginosas (ANTILLE et al., 2013; SENJOBI et al., 2013).

Outro fator importante no cultivo do morangueiro é a disponibilidade de água. A escassez dos recursos hídricos e a seca, principal estresse ambiental, incentivam a utilização de estratégias que reduzam o uso de água na agricultura (GRANT et al, 2010). Assim, são necessárias pesquisas relacionadas à adubação e à irrigação adequadas para suprir as necessidades nutricionais e hídricas do morangueiro e melhorar o potencial nutricional e funcional dos frutos, sem, no entanto, prejudicar o desenvolvimento da planta, e que contribuirão não somente para o conhecimento científico, mas também para o setor de produção agrícola, atendendo os consumidores mais exigentes, uma vez que tem aumentado a busca por alimentos com características benéficas à saúde e livres de produtos químicos.

Com esse intuito, essa dissertação intitulada “Nutrição e aporte hídrico: alterações bioquímico-fisiológicas e moleculares em morangos cv. Camarosa foi desenvolvida através de uma parceria entre a Universidade Federal de Pelotas (UFPel) e a Embrapa Clima Temperado (CPACT/Pelotas/RS), sob a orientação da equipe de trabalho constituída por Prof. Dr. Cesar Valmor Rombaldi, Dr. Rafael da Silva Messias, Pesquisador Dr. Carlos Augusto Posser Silveira e Prof.ª. Dra. Josiane Freitas Chim.

A estruturação da dissertação é constituída dessa introdução geral, abordando de forma sucinta os temas que serão abordados nessa dissertação e justificativa do estudo; uma revisão bibliográfica, mais ampla, abordando o conceito de biofortificação com ênfase em estresse abiótico, especificamente em estresse hídrico, e descrevendo as principais respostas e consequências desse estresse com foco no metabolismo dos fenilpropanóides, objetivando posteriormente realizar uma reestruturação da mesma em uma revisão bibliográfica mais aprofundada adicionando diferentes estresses abióticos com foco no potencial funcional e nutricional dos alimentos visando uma publicação em um periódico internacional;

matérias e métodos e resultados e discussão foram divididos em dois artigos; e por conseguinte, as conclusões do estudo e as referências que deram suporte ao projeto.

Os artigos desse estudo foram: “Artigo 1”: *“Effect of an alternative organomineral fertilizer on yield and quality of strawberry fruits”*, desenvolvido para avaliar o potencial biofortificante de uma adubação chamada aqui de “alternativa”, que propõe a utilização de fontes de nutrientes natural/alternativo, avaliando juntamente seu efeito em variáveis agronômicas e desenvolvimento das plantas de morangueiro, em comparação com a adubação convencional, que utiliza fontes solúveis dos nutrientes. No “Artigo 2”: “E*ffect of drought stress in photosyntetic variables, yield and antioxidant compounds in strawberry fruits”*, avaliou-se o comportamento fisiológico das plantas e qualidade final dos frutos submetidos a diferentes níveis de estresse hídrico associado à adubação alternativa.

É importante ressaltar que para alcançar o desenvolvimento adequado do experimento, bem como confiabilidade dos resultados, diversas avaliações e testes foram realizados antes da implantação do experimento e também testes nos métodos e tratamento dos dados (essas informações não constam na dissertação, pois constituíram os ensaios preliminares). Os apêndices de A a K apresentam uma sequencia da condução do experimento, bem como dados adicionais utilizados nessa dissertação.

Os resultados desse estudo permitiram propor uma adubação com potencial biofortificante, sem alteração marcante na produtividade dos frutos. O comportamento fisiológico do morangueiro em resposta à aplicação de estresse hídrico também foi monitorado. No entanto, ainda é necessário encontrar níveis moderados de estresse que não prejudiquem significativamente a produção da cultura e simultaneamente exerçam um efeito biofortificante.

1.1. Hipótese

Morangueiros cultivados com adubação alternativa e estresse hídrico moderado resultam na obtenção de frutos com maior qualidade pela presença de compostos com potencial nutricional e funcional sem inviabilizar o desenvolvimento das plantas e produção de frutos.

1.2. Objetivo geral

Avaliar o efeito biofortificante de adubação alternativa e estresse hídrico moderado em plantas de morangueiro, através de variáveis bioquímico-fisiológicas e moleculares.

1.2.1. Objetivos específicos

- Avaliar variáveis agronômicas em folhas de morangueiro durante o ciclo da cultura, sendo elas: taxa de assimilação de gás carbônico, condutância estomática, taxa de transpiração, produção de frutos e biomassa vegetal e no solo pela condutividade elétrica;
- Avaliar variáveis bioquímicas relacionadas ao acúmulo de compostos que contribuem para o potencial funcional de vegetais: compostos fenólicos, antocianinas, ácido L-ascórbico e atividade da enzima fenilalanina amionia liase e enzimas oxidantes, e;
- Avaliar o acúmulo relativo de transcritos relacionados à rota metabólica dos fenilpropanóides.

2. Revisão Bibliográfica

2.1. Biofortificação

Um número significativo de pessoas (em torno de 850 milhões) sofrem alguma forma de desnutrição e/ou desequilíbrios nutricionais. Baixos teores de nutrientes no solo, fatores antinutricionais, como os metais pesados tóxicos que reduzem a absorção de micronutrientes pelo organismo humano, além da baixa diversidade das dietas são as principais causas de desnutrição (MORAES et al., 2012). A desnutrição e a recomendação de ingestão de compostos com potencial antioxidante, importantes para a prevenção de doenças crônicas, têm estimulado a busca por alternativas que melhorem a oferta destes compostos, tanto com potenciais nutricionais quanto funcionais (CONNOLLY, 2008; JOHNS e EYZAGUIRRE, 2007). Adicionalmente, fatores climáticos e a degradação de recursos naturais, bem como o aumento populacional, também justificam a busca por estratégias que garantam uma melhor qualidade dos alimentos produzidos, visto que a Organização para a Alimentação e Agricultura (FAO) estima que até 2050 a população mundial deva atingir 9,1 bilhões (FAO, 2009). Essa projeção, associada à

melhoria ao acesso a alimentos, representará um desafio importante para a agricultura: produzir mais sem aumentos proporcionais de área cultivada, e produzir alimentos diferenciados, seja sob o ponto de vista nutricional ou funcional, seja pelo sistema de produção.

Tecnologias clássicas foram utilizadas por muitos anos e ainda são aplicadas na tentativa de garantir o fornecimento de alimentos à população mundial, como o uso de fertilizantes solúveis, agroquímicos e irrigação; porém, em alguns casos, essas técnicas apresentam limitações, e podem prejudicar a qualidade dos alimentos (GROOTE, 2012). Assim, a biofortificação de culturas agrícolas constitui-se em uma alternativa para agricultura, sendo uma ferramenta para melhorar a qualidade dos alimentos sem, no entanto, prejudicar a produtividade (HOTZ, 2013; ZHU et al., 2007; MORAES et al., 2012). As estratégias de melhoramento convencional, transgenia e fertilização são três estratégias comumente utilizadas para se atingir esses objetivos (SALTZMAN et al., 2013).

Durante muitos anos o melhoramento convencional tinha como foco o incremento do rendimento das culturas agrícolas, aumentando a resistência às pragas e doenças e a tolerância a estresses abióticos como estresses osmóticos. Mais recentemente, o foco vem sendo dirigido para melhorar as concentrações de micronutrientes, vitaminas e compostos bioativos (WINKLER, 2011). Variedades com níveis elevados de vitamina ou mineral são cruzadas por várias gerações para produzir plantas que têm as características agronômicas e de nutrientes desejadas. O principal exemplo de bioforticação por melhoramento convencional é a batata doce laranja (“Orange sweet potato”) melhorada a partir da seleção de variedades com altos níveis de pró-vitamina A (SALTZMAN et al., 2013).

A transgenia, é vantojosa quando o nutriente não existe naturalmente em determinada cultura ou quando quantidades suficientes de micronutrientes biodisponíveis não podem ser eficazmente produzidos para o cultivo. No entanto, uma vez que uma linha transgênica é obtida, vários anos de melhoramento convencional são necessários para assegurar que os genes foram estavelmente herdados. Além disso, muitos países não têm quadros legais para permitir a liberação e comercialização destas variedades. Esta abordagem tem sido utilizada, por exemplo, para aumentar o teor de ferro (Fe) e zinco (Zn) em alimentos, dois nutrientes minerais que são constantemente insuficientes nas dietas dos humanos (CURIE e BRIAT, 2003; PALMGREN et al., 2008). Vasconcelos et al. (2003)

observaram um aumento de 3 a 4 vezes no teor de Fe em arroz, devido à superexpressão de ferritina, uma proteína responsável pelo armazenamento de ferro. O exemplo mais típico de biofortificação através desta ferramenta é o arroz dourado (“Golden Rice”), no qual a biossíntese de carotenóides foi manipulada a fim de induzir a produção de β-caroteno (pró-vitamina A) para ajudar no combate a deficiência desta vitamina (PAINE et al., 2005).

A fertilização é uma prática relativamente simples e com resultados imediatos, esta estratégia pode ser utilizada para fortalecer as culturas com elementos minerais e não com nutrientes orgânicos (por exemplo, vitaminas), que são sintetizados pela própria planta. Além disso, a composição do solo, mobilidade mineral no solo e na planta e o seu local de acúmulo são fatores variáveis (HIRSCHI, 2009). Logo, a aplicação de fertilizantes contendo micronutrientes minerais essenciais não pode ser vista como uma abordagem universal para melhorar os níveis de micronutrientes (WHITE; BROADLEY, 2009).

Além dessas três estratégias, estudos têm indicado a possibilidade de indução de estresses abióticos em níveis moderados, os quais poderiam resultar no incremento do conteúdo de compostos com potencial funcional, influenciando positivamente na qualidade do alimento sem acarretar prejuízos ao desenvolvimento das plantas (KIM et al., 2008; COGO et al., 2011), podendo esta ser uma estratégia eficaz de biofortificação de compostos com potencial antioxidante.

2.2. Estresses abióticos

Fatores ambientais como seca, calor, salinidade, radiação UV em excesso (WANG e FREI, 2011), ozônio, luz, excesso ou deficiência de nutrientes e presença de metais pesados caracterizam um estresse abiótico. Esses fatores, de forma extrema, causam uma intensa reorganização do metabolismo vegetal, reduzindo a atividade e crescimento celular (LICHTENTHALER et al., 1998), que afeta os níveis ideais de desenvolvimento podendo levar à morte da planta (CRAMER et al., 2011). Para adaptação a situações contínuas de estresses abióticos em seu ambiente natural, os vegetais possuem mecanismos complexos para captação de sinais e modulação de respostas, na tentativa de retornar à condição homeostática mais próxima do ideal (SOARES e MACHADO, 2007; FUJITA et al., 2006).

As respostas ao estresse dependem da intensidade da condição exposta e passam por um processo que basicamente pode ser dividido em quatro fases, conforme ilustrado na figura a seguir.

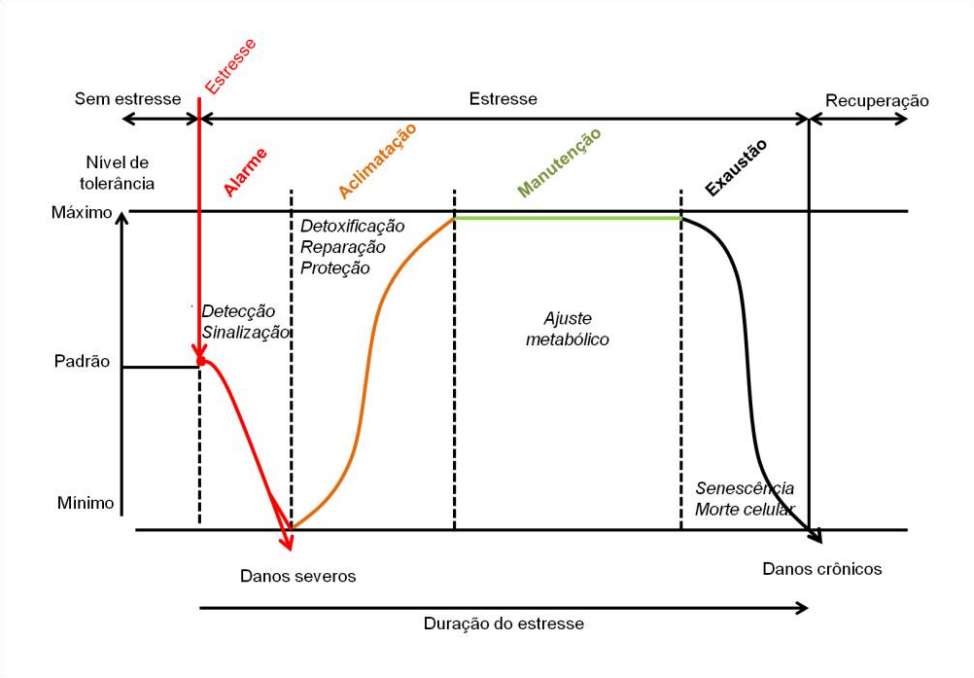

Figura 1. Fases geradas durante a resposta das plantas em situações de estresses (alarme, aclimatação, manutenção e exaustão). Figura adaptada de Cabane et al., (2012).

Quando não há a exposição a estresses, as plantas estão em condição fisiológica normal, ou seja, em homeostase; porém, quando submetidas a situações de estresse, as plantas entram em estado de alarme, onde os níveis de tolerância são mínimos e ocorre alteração dos níveis fisiológicos ideais da planta, acarretando em possíveis danos severos, induzindo vias de sinalização do estresse. A partir de então, o nível do estresse e capacidade de tolerância da planta serão decisórios para as respostas nos processos posteriores, pois podem ocorrer também além dos danos severos à senescência da planta. Caso a planta possua níveis de tolerância suficientes para suportar essa situação é então seguida para a segunda fase associada à ativação de mecanismos de reparação, proteção e detoxificação, chamada de aclimatação, onde a planta apresentará uma condição fisiológica distinta da homeostase da planta antes do estresse, mas que pode se tornar estável, conduzindo a uma fase de manutenção. Nessa terceira fase, ocorrem reestruturações/ajustes profundos no metabolismo podendo ser estabilizada e mantida em nível de estresse prolongado. Se nessa terceira fase a planta não resistir, por uma intensidade demasiada de estresse, ocorre declínio da vitalidade da

planta gerando danos crônicos e consequentemente morte celular, caracterizando a quarta fase denominada fase de esgotamento. No entanto, se o estresse é cessado ou removido antes da planta entrar em senescência, o metabolismo vegetal será conduzido então para uma fase de recuperação (LICHTENTHALER, 1998; KOZOVA et al, 2011; CABANE et al., 2012).

Dentre as respostas desenvolvidas pelas plantas em situações de estresse pode ocorrer o aumento da atividade de culturas reativas de oxigênio (ROS), compostas por: radical superóxido ($O_2^{\bullet-}$), peróxido de hidrogênio (H_2O_2), oxigênio singleto ($^1O_2$), radical perhidroxil ($HO_2^{\bullet-}$), radical hidroxila ($OH^{\bullet}$), hidroperóxidos (ROOH), radical alquilperoxila ($ROO^{\bullet}$) e o radical alcoxila ($RO^{\bullet}$) (GILL e TUTEJA, 2010), bem como espécies reativas de nitrogênio: óxido nítrico ($NO^{\bullet}$), peroxinitrito ($ONOO^{\bullet}$), espécies reativas de enxofre: radical tiíla ($RS^{\bullet}$) (é uma denominação genérica para um grupo de radicais com o elétron desemparelhado residindo no enxofre, o qual é formado quando um grupo tiol (RSH) reage com uma espécie radicalar ou então com metais de transição), e por fim miscelâneos e metais, como Fe, Cu, Mn que catalisam reações de radicais livres (VASCONSCELOS et al., 2007; BIANCHI e ANTUNES, 1999; VELLOSA et al., 2007). De uma forma geral, quando o aumento de ROS ultrapassa a capacidade antioxidante da planta, caracterizando um estado de estresse oxidativo, a planta ativa mecanismos de defesa frente a essas substâncias, onde são ativados e sintetizados compostos com capacidade antioxidante com o objetivo de retornar ao seu equilíbrio. Como exemplo de compostos com esse potencial, têm-se: as enzimas superóxido dismutase (SOD), catalase (CAT), ascorbatoperoxidase (APX), glutationaredutase (GR) e os compostos ácido ascórbico, glutationa e compostos fenólicos em geral (GONG et al., 2013).

Entre a diversidade de metabólitos produzidos pelas plantas como forma de defesa, os metabólitos especializados desempenham um significativo papel na adaptação a essas condições de estresse, já que os vegetais possuem grande capacidade para síntese desses compostos sendo componentes integrais de mecanismos de defesa (NCUBE et al., 2012; RAMAKRISHNA e RAVISHANKAR, 2011).

No entanto, a síntese desses compostos dependerá do estádio de desenvolvimento da planta, das condições a que esta está exposta, tecido e órgãos

afetados, onde enzimas envolvidas em diferentes vias metabólicas serão ativadas ou não (NCUBE et al., 2012; CRAMER et al., 2011), afetando a planta positivamente ou negativamente. Os efeitos positivos e negativos podem auxiliar no desenvolvimento de estratégias para o cultivo em ambientes adversos e para obtenção de culturas biofortificadas (WANG e FREI, 2011).

2.3. Estresse hídrico

Dentre os fatores ambientais que caracterizam os estresses abióticos, o fornecimento de água nas irrigações é um fator limitante ao desenvolvimento vegetal. Situações de estresse hídrico ocorrem quando há uma redução da disponibilidade de água no solo ou sua perda contínua (RAMAKRISHNA e RAVISHANKAR, 2011). Assim, há a necessidade de desenvolver estratégias que melhorem a eficiência do uso da água, devido a mudanças climáticas e a sua crescente escassez (LIU et al., 2007).

A água é a molécula central da maioria dos processos fisiológicos das plantas, sendo o principal meio de transporte de nutrientes e metabólitos (RAHMAN e HASEGAWA, 2011). Logo, a quantidade de água requerida pelas plantas em praticamente todos os estádios de desenvolvimento deve ser suficiente para suprir essa necessidade. Se esse aporte hídrico não for adequado, poderá influenciar em diversos processos fisiológicos e morfológicos dos vegetais, levando a provável redução do tamanho, aumento da suscetibilidade ao ataque de patógenos, alteração nos hormônios, além de redução da área foliar e da produtividade na produção (MEDEIROS et al., 2007), sendo este considerado o maior efeito negativo do estresse hídrico (FIOREZE et al., 2011).

As respostas geradas frente a condições de seca dependerão principalmente do nível de estresse ocasionado. Em condições moderadas, a planta normalmente induz a regulação de captação da perda de água, permitindo a manutenção de água nas folhas de forma tal que a capacidade fotossintética mostre pouca ou nenhuma alteração. Em contrapartida, níveis severos de estresse hídrico levam a alterações desfavoráveis de fotossíntese e crescimento. Portanto, baseado na presença ou ausência de água, são ativados diferentes mecanismos de respostas (YORDANOV et al., 2013).

Dentre os mecanismos de repostas estão o aumento das raízes, a redução da absorção de radiação luminosa e evaporação na superfície foliar e a diminuição da

perda de água através da redução da condutância (cuticular e estomática) epidérmica (HARB et al., 2010), levando ao desequilíbrio hormonal, uma alteração fotossintética resultante do desequilíbrio entre a captura de luz e sua utilização, sendo criado o desequilíbrio entre a geração e utilização dos elétrons (RAHMAN e HASEGAWA, 2011), alteração na atividade de enzimas responsáveis por metabolismos de defesa, modulação do sinal de transdução, e alteração da expressão de genes relacionados (ASHRAF et al., 2011), além do acúmulo de solutos antioxidantes (SILVA et al., 2002).

Neste contexto, a busca por alternativas que reduzam o uso da água na agricultura pode ser justificada não somente pela escassez dos recursos naturais e mudanças climáticas, mas também pelo incremento da qualidade funcional e nutricional dos alimentos, desde que sem afetar o rendimento das culturas. Ou, ocorrendo redução no rendimento, que a mesma seja compensada pelos benefícios que a estratégia desenvolvida possa trazer (STEFANELLI et al., 2010). Assim, esta pode ser uma estratégia eficaz de biofortificação, pois estresses moderados induziriam a produção de compostos antioxidantes acarretando um aumento da tolerância destas plantas a estresses subsequentes, uma vez que seu metabolismo já estaria direcionado à produção desses metabólitos especializados, e ao mesmo tempo proporcionando a biofortificação do alimento gerado através do aumento no seu potencial antioxidante (MESSIAS et al., 2013).

2.4. Metabolismo especializado com ênfase nos compostos fenólicos

Os metabólitos especializados são referidos como compostos que não possuem um papel fundamental na manutenção de processos vitais nas plantas, mas que são importantes na interação com o seu ambiente de adaptação, assim como para defesa frente a agressores bióticos e abióticos. Uma grande variedade desses metabólitos é sintetizada a partir de metabólitos primários (por exemplo, hidratos de carbono, lipídeos e aminoácidos) em plantas superiores. Entretanto, além de desenvolverem função de defesa em algumas situações dos vegetais, pela sua capacidade antioxidante, estes são responsáveis também pela pigmentação, sendo utilizados como fontes únicas para aditivos, aromatizantes (RAMAKRISHNA e RAVISHANKAR, 2011).

Estes metabólitos representam adaptações evolutivas que os vegetais desenvolveram para responder a diferentes condições a que foram expostos, como

estresses bióticos e abióticos (PICHERSKY e LEWINSOHN, 2011). O metabolismo especializado se divide em três abrangentes classes de compostos, sendo elas: terpenos, alguns compostos nitrogenados e compostos fenólicos (BERGAMASCHI, 2010). Os terpenos são derivados do ácido malônico ou do piruvato 3 –fosfoglicerato e possuem função no crescimento e desenvolvimento das plantas, são essências às membranas celulares (esteróis), atuam como pigmentos acessórios na fotossíntese (carotenóides), conferindo atividade antioxidante através da interação com radicais livres (LICHTENTHALER, 1999). A classe de compostos nitrogenados é derivada de aminoácidos aromáticos (triptofano e tirosina) e inclui alguns compostos que atuam na defesa das plantas contra os parasitas e herbívoros, como os alcalóides e os glicosídeos cianogênicos (MITHEN et al., 2000). Os compostos fenólicos apresentam significativa importância por participarem de diferentes processos, como lignificação, pigmentação, crescimento, resistência contra predadores e patógenos, estresses ambientais e polinização (FRAGA et al., 2010).

2.4.1. Compostos fenólicos

O incremento nos teores dos compostos fenólicos em frutos tem sido alvo de diversos estudos (ZHENG et al., 2007; FAN et al., 2012; SHIN et al., 2007) em virtude de seus efeitos benéficos devido a sua estrutura química, pelo sequestro de radicais livres no organismo humano, além de auxiliarem no combate ao envelhecimento precoce das células, a suscetibilidade a infecções, aumento da atividade antiinflamatória, dentre outros (FRAGA et al., 2010).

Quimicamente os compostos fenólicos possuem um ou mais grupos hidroxilas ligados a um anel benzeno e são sintetizados através dos precursores acetato e chiquimato (fenilpropanóides). Os compostos fenólicos que são produzidos pela via dos fenilpropanóides contribuem para a pigmentação dos frutos e defesa em condições de estresses (SINGH et al., 2010). Quando sintetizados por essa via, a enzima fenilalanina amônia liase (FAL) catalisa a reação de entrada nessa rota através da desaminação do aminoácido L-fenilalanina em ácido cinâmico, seguida de uma série de reações enzimáticas, envolvendo flavonol sintase (FLS), que atua na formação de flavonóides, antocianidina sintase (ANS), diretamente envolvida na formação de antocianidinas, e UDP flavonóide glicosiltransferase (UFGT), responsável pela síntese de antocianinas (ALMEIDA et al., 2007) (figura 2).

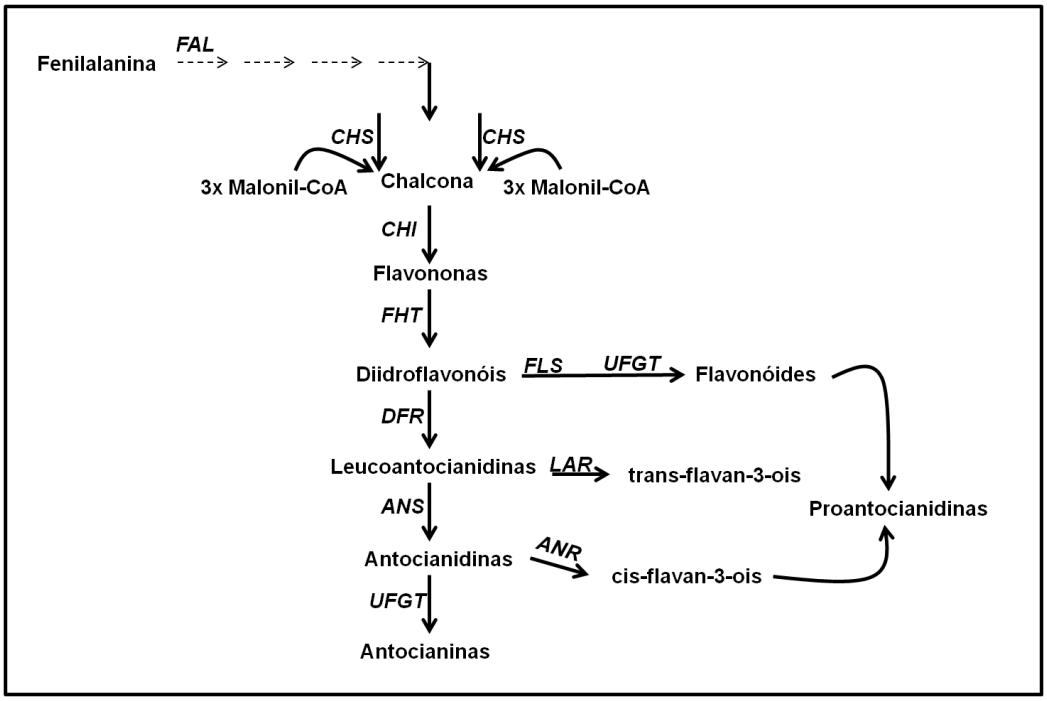

Figura 2. Esquema simplificado representando a rota metabólica de fenilpropanóides. Figura adaptada de Almeida et al., (2007).

Os compostos fenólicos são classificados como flavonóides e não flavonóides. No entanto, os flavonóides representam a maior parte desses compostos, possuem baixo peso molecular e são encontrados naturalmente nas plantas, sendo responsáveis pela coloração de alguns frutos, flores e folhas (VOLP et al., 2007).

Os flavonóides são os pigmentos mais comuns depois da clorofila e dos carotenóides. Devido a sua cor atrativa contribuem como sinais visuais para os insetos durante a polinização e a adstringência de alguns compostos representa um sistema de defesa contra insetos nocivos às plantas. Além disso, atuam como catalizadores da fotossíntese e protetores contra espécies reativas de oxigênio (STALIKAS, 2007). Estruturalmente possuem um esqueleto comum de difenil piranos composto por anéis fenil ligados através de um anel pirano (heterocíclico). Assim, se dividem em diferentes classes, dentre elas estão flavanóis, flavonóis, flavonas, isoflavonóides, flavononas e antocianinas. Essa gama de classes é devida a diferenciação da substituição dos anéis, modificações que tais compostos podem sofrer, ao nível de oxidação e às variações no esqueleto carbônico básico, causada pelas reações de oligomerização, hidroxilação, metilação, glicosilação, entre outras (COUTINHO et al., 2009; SILVA, 2004). A atividade antioxidante dos flavonóides

depende da propriedade redox de seus grupos hidrofenólicos e da relação estrutural entre as diferentes partes da estrutura química (ANGELO; JORGE, 2007). Os possíveis benefícios à saúde de uma dieta com alimentos ricos em compostos fenólicos dependem da sua absorção e metabolismo, que são determinados pela estrutura química, como a conjugação com outros fenólicos, grau de glicosilação, acilação, tamanho molecular, solubilidade, hidroxilação e metilação (BALASUNDRAM et al., 2006)

Subclasse dos flavonóides, as antocianinas são um grupo de compostos com significativa importância, pois são responsáveis pela maioria das cores vermelha, rosa, roxa e azul observadas nos vegetais. Estruturalmente são constituídos por glicosídeos que apresentam açúcares ligados a sua estrutura no carbono três do anel pirano, sendo a estrutura básica denominada de cátion 2-fenilbenzopirílio. Sem a presença dos açúcares em sua estrutura, as antocianinas são conhecidas como antocianidinas. A cor das antocianinas é influenciada pelo número de grupos hidroxila e metoxila da antocianidina, pela presença de ácidos aromáticos esterificados ao esqueleto principal e o pH do vacúolo no qual tais compostos estão armazenados.

As antocianinas sofrem a reação de co-pigmentação, pela ligação de moléculas que estabilizam a estrutura, tornando-a complexa com maior resistência a desprotonação (PADAYACHEE et al., 2012). As antocianinas mais comumente encontradas são baseadas nas antocianidinas: pelargonidina, cianidina, malvidina, delfinidina, petunidina e peonidina. Como propriedade também apresenta atividade antioxidante, antimicrobiana, anti-inflamatória e anticancerígena, pela eliminação de ROS, inibindo ou retardando reações indesejáveis (PADAYACHEE et al., 2012). Nas dietas humanas são encontradas normalmente associadas com frutas, principalmente em morango, amora, mirtilo, framboesa, uvas e groselhas, muito embora também sejam encontradas em cereais, raízes e legumes (FERNANDES et al., 2013).

2.5. Morango

O morangueiro pertence à família das Rosaceaes, do gênero *Fragaria*, abrangendo mais de 600 cultivares comerciais, que diferem no sabor, tamanho, textura e composição química das frutas que, botanicamente, são pseudofrutos (ANTUNES et al., 2010; PADULA et al., 2013). Encontra-se como uma das mais

populares frutas devido ao seu alto consumo por suas características sensoriais, nutricionais e funcionais apreciáveis. Seu aroma, por exemplo, é caracterizado como um dos mais complexos, sendo possível encontrar cerca de 350 compostos voláteis (VANDENDRIESSCHE et al., 2012). É considerado como fonte de compostos fenólicos, sendo as principais classes: flavonóis (quercetina, kaempferol e miricetina), taninos hidrolisáveis (elagitaninos), ácidos hidroxibenzóicos (ácido gálico e elágico), ácido hidroxicinâmico (p-cumarico), catequina, epicatequina e antocianinas (principalmente pelargonidina 3-glicosídeo), que é o pigmento mais importante nesta cultura. Todos esses compostos variam conforme fatores edafoclimáticos e genotípicos (PINELI et al., 2011; ERKAN et al., 2008). Na composição química desse fruto, também estão presentes minerais e vitaminas C e do complexo B (ROCHA, 2010). Assim, a atividade antioxidante total do morango bem como de seus subprodutos será dependente do conteúdo de compostos fenólicos, vitaminas e antocianinas presentes (CAPOCASA et al., 2008).

O cultivo do morangueiro é caracterizado pela sua elevada rentabilidade e emprego intensivo de mão-de-obra (ANDRIOLO et al., 2009) que, juntamente com a diversidade de opções de comercialização e processamento do morango, são fatores que levam ao grande interesse pelo seu cultivo (MENDONÇA, 2011). Para o cultivo é necessário fornecer quantidades de água ideais conforme o desenvolvimento das plantas, pois elas possuem sistema radicular de pouca profundidade, área foliar extensa e frutos de elevado conteúdo de água (KLAMKOWSKI e TREDER, 2006).

Alguns estudos vêm apresentando o comportamento de folhas e frutos do morangueiro frente a condições reduzidas de disponibilidade de água. Johnson et al., (2009) avaliaram respostas de dez cultivares de morango em condições limitadas de água, visualizando uma interação significativa entre cultivar e tratamento de estresse hídrico na variável produtividade, indicando portanto que as cultivares diferem na sua resposta ao estresse hídrico. O mesmo efeito dependente de diferentes cultivares foi observado por Bordonaba e Terry (2010), onde as cultivares Christine, Elsanta, Florence, Sonata e Simphony apresentaram diferentes respostas frente ao estresse hídrico aplicado. As cv. Elsanta, Sonata e Symphony mostraram uma maior redução no tamanho dos frutos, acompanhado por um aumento significativo no teor de matéria seca, e, consequentemente, à concentração de açúcares, enquanto ‘Florence’ e ‘Christine’ não apresentaram variações

significativas no peso dos frutos ou de qualquer analito analisado. Os autores sugerem que a redução de água na irrigação entre a floração e a colheita pode ser uma técnica viável para aumentar a concentração de compostos relacionados ao sabor nas cultivares Elsanta, Sonata e Symphony.

Ghaderi e Siosemardeh (2011) avaliaram respostas fotossintéticas em duas cultivares de morango (Kurdistan e Selva), cultivadas em condições de déficit hídrico. As variáveis: taxa de assimilação líquida de gás carbônico, a condutância estomática e a transpiração diminuíram nas condições do estudo. Além disso, constataram que a quantidade de carboidratos aumentou sob estresse hídrico severo. Este estudo revelou também que o estresse hídrico moderado afeta a troca gasosa, enquanto o estresse severo afeta clorofila, prolina e os níveis de carboidratos solúveis.

As respostas antioxidantes de morangos sob estresse hídrico também já foram estudadas. Neocleous et al., (2012) estudaram essas respostas antioxidantes em morangos cv. Camarosa e observaram um aumento na atividade da peroxidase (POD), sugerindo que as plantas de morango possuem mecanismos específicos para desintoxicar espécies reativas de oxigênio em condições de estresse hídrico.

No entanto, pesquisas relacionadas às respostas de folhas e frutos morangueiro em condições de estresse hídrico limitam-se a abordar principalmente variáveis agronômicas e de qualidade, de forma individual, o que dificulta o entendimento dos mecanismos de repostas. Estudos abordando respostas agronômicas, bioquímicas e fisiológicas, de forma conjunta, irão contribuir com o esclarecimento do comportamento global do morangueiro em condições de déficit hídrico. Além disso, o conhecimento de níveis moderados de estresse, que não prejudiquem a produção da cultura e simultaneamente provoque um incremento no potencial nutricional e funcional dos morangos, irá contribuir para a biofortificação dessa cultura, a baixo custo e com a adicional economia dos recursos hídricos.

3. Artigo 1 – “Effect of an alternative organomineral fertilizer on yield and quality of strawberry fruits”

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EFFECT OF AN ALTERNATIVE ORGANOMINERAL FERTILIZER ON YIELD AND QUALITY OF STRAWBERRY FRUITS

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Abstract

Strawberry crop plays a major economic role worldwide. Its cultivation is currently performed using soluble fertilizers that may result in negative environmental effects, soil poorness and low accumulation of essential minerals for human health. Therefore, alternative and natural source of fertilizers are of great importance. Organomineral fertilizers obtained from industrial wastes may be a promising source to use in agriculture. Therefore, we compared two fertilizations, a conventional fertilization based on soluble nutrient sources, and an alternative fertilization based on rock powders (granodiorite gneiss, natural phosphate) and oilseed cake (tung press cake), regarding yield and quality of strawberry fruit 'Camarosa'. The results indicate that the alternative fertilizer evaluated has potential to be used in agriculture by increasing fruit yield and improving the accumulation of nutrients and compounds with functional potential in strawberry fruits as anthocyanins, L-ascorbic acid and some phenolic compounds (gallic acid, Hydroxybenzoic acid and ellagic acid).

Key words: *Fragaria x ananassa*, soluble fertilizer, alternative fertilizer, yield, fruit quality.

1. INTRODUCTION

The strawberry fruits (*Fragaria* x *ananassa*) are one of the richest sources of natural antioxidants, such as vitamins, anthocyanins and flavonoids. It is also well known by its flavor and aroma and by the high content of minerals (Erkan, Wang & Wang, 2008).

Strawberry crop plays an important socio economic role in Brazil and worldwide. Approximately 4.5 million tons of strawberry fruits are produced per year around the word, and approximately 130 thousand tons are produced in Brazil (Fao, 2013). However, the production of strawberry plants is hampered because it requires high content and availability of macronutrients and micronutrients for fruits yield (Singh, Sharma, Kumar, Gupta & Patil, 2008). Therefore, plant production systems play a key role for improving plant growth and they affect the yield, size and chemical composition of fruits (Fan et al. 2012). The soluble fertilizers are the most widely used for the purpose of strawberry production; however, these fertilizers are based primarily on macronutrients (nitrogen - N; phosphorus – P; and potassium - K) to increase productivity. Therefore, their intensive use and the extraction rate of these nutrients by crops may result in environmental damages such as soil depletion (Lal, 2009), increasing of soil salinity and leaching, that could contaminate the soil and groundwater (Sors, Ellis & Sant, 2005). Consequently, the plants may show symptoms of phytotoxicity and micronutrient deficiency (McCauley, Jones & Jacobsen, 2008), leading to a low accumulation of essential minerals for human health in the fruits (Welch & Graham, 2004).

Also, most of the raw materials used as inputs for soluble fertilizers production are from finite sources and in many cases (including Brazil) have been acquired via import, resulting in high costs to the productive sector. Thus, the search for natural

sources and environmentall friendly fertilizers able to improve absorption and/or translocation of minerals via xylem, enhance vegetative growth and yield, and improve the functional and nutritional quality of food is a worldwide tendency (Gómez-Galera et al. 2010). In this context, several studies have related the use of by-products from industrial and mining processes as a natural source for fertilization, representing a local low-cost strategy for the disposal of these by-products. (Shivay, Krogstad & Singh, 2010; Silva et al. 2012).

The production of biodiesel and other chemical products from vegetal sources as a renewable energy supply has increased considerable due to the search for petrochemicals alternatives. Therefore, the production of by-products derived from this activity has also increased. The tung (*Vernicia fordii*) press cake is a by-product from the oil extraction of tung seeds to produce biodiesel and other industrial products that may be a potential organic source of nutrients for plant development (Wilkins, 2012; Gruszynski, 2002). The use of organic residues as fertilizer has shown positive effects on productivity as well as an increase in the quality of different crops (Kashif, Yaseen, Arshad & Ayub, 2004).

The mining activity to obtain products for construction, building or even to petroleum extraction has also resulting in the generation of wastes, causing a notorious environmental problem that must be prevented. Thus, the use of rocks in agriculture, also known as “stone meal”, may represent an important approach towards sustainable development. These rocks are finely ground (< 0.3 mm) to be applied to the plants and they are generally selected as sources of P, K, calcium (Ca), sulphur (S) and magnesium (Mg), but acting as a matrix that are also source of several micronutrients and trace elements that are essential for plant nutrition (Shivay et al. 2010; Silva et al. 2012). These rock powders have also the beneficial of

gradual release of these nutrients to the soil and consequently balancing the absorption rate to the plants when compared to the soluble fertilizers, contributing for the establishment of the soil fertility (Elsaesser, Kunz & Briemle, 2008).

Therefore, this study aimed to compare two fertilization strategies, one with soluble nutrient sources (conventional fertilization), and another with natural/alternative sources based on rock powders and tung press cake regarding yield and quality of strawberry fruits.

2. MATERIAL AND METHODS

2.1. Plant material and experimental conditions

The experiment was conducted in a greenhouse at Brazilian Temperate Agriculture Research (Embrapa) (Pelotas/RS/BR) from May to December 2012. Strawberry seedlings 'Camarosa' were transplanted and grown in 9 L pots, containing a mixture of soil and vermiculite (3:1). The experimental design was completely randomized with four replications and two treatments. Treatments were arranged in one-factor scheme. The treatments were: SF-soluble fertilizer, constituted by soluble sources of N, P and K (urea, triple superphosphate and potassium chloride, respectively); and AF- alternative fertilizer, constituted by natural/alternative sources of NPK (tung press cake, natural phosphate and granodiorite, respectively). The quantity of each component of both fertilizers was calculated to obtain the equivalent of 120 kg ha^{-1} N, 260 kg ha^{-1} P_2O_5 and 200 kg ha^{-1} K_2O, according to the fertilizer recommendations for this crop based on the preliminary analysis of the soil (Supplementary data 1). Moreover, 2,200 kg/ha of limestone was included in both treatments to correct the soil pH according to the

recommendation for this crop (CQFS, 2004). The irrigation was performed by daily dripping, and the irrigation volume was adjusted weekly, according to the evapotranspiration value of the culture, as proposed by Marouelli, Silva & Silva (2008).

Two sampling times of mature fruits were performed during the crop productive phase (ST1 and ST2, respectively), as shown in Figure 1. The fruits were weighed to obtain the yield value and immediately frozen with liquid nitrogen. The samples were stored in ultrafreezer at -80 °C and lyophilized to perform the fruit quality analysis.

2.2. Photosynthetic variables of strawberry leaves

The CO_2 assimilation rate (A), total water vapor conductance (GH_2O) and transpiration rate (E) of the plants were monitored with a portable gas exchange fluorescence system infrared gas analyzer (IRGA) (Heinz Walz GmbH, GFS 3000 model). Regular measurements were performed during the crop cycle in novel full-developed leaves, totalizing five measurements (M1 to M5, as presented in Figure 1). Previous curves were performed to determine the amounts of CO_2 and light for use in the measurement.

2.3. Soil electrical conductivity

The soil electrical conductivity was determined with a conductivimeter (Tecnal, TEC-4MPP model) in samples collected randomly from the pots, during the crop cycle, totalizing six measurements (M1 to M6, as presented in Figure 1). Representative soil samples from each biological replicate were collected and oven

dried, and the electrical conductivity was determined on the supernatant of aqueous soil extracts obtained in a mixture of soil: distilled water (1:5).

2.4. Yield of strawberry fruits and fresh and dry plant biomass

For determination of crop yield, fruits in commercial stage of maturation (full red, characterized by developmental stage 8, according to Jia et al. 2012) were collected and weighed during all the crop cycle. The results were showed as the cumulative yield up to ST1, cumulative yield from ST1 to the ST2 and the total yield. Fresh and dry plant biomass (FB and DB) were determined at the end of the crop cycle by weighing the aerial portion of the plant, before and after drying in an oven with air circulation at 60 °C, respectively.

2.5. Mineral composition of strawberry fruits

The mineral composition was determined in lyophilized samples. The content of P, K, Ca, Mg, cooper (Cu), manganese (Mn) and boron (B) was quantified by atomic absorption spectrometry (AAS) (Varian™ AA240FS, Santa Clara, CA) from 250 mg of sample digested with 1 mL of H_2O_2 and 5 mL of HNO_3 in microwave, according to Silva (2009). Mean values were expressed as g of mineral per kg of fruit in dry weight (DW). The analyses were performed in four biological replicates and three analytical replicates.

2.6. Total phenolic compounds, total anthocyanins and total antioxidant activity of strawberry fruits

The total phenolic compounds were quantified by the method from Swain, Hillis (1959). Briefly, 0.5 g of lyophilized sample was extracted with 20 mL of

methanol P.A. Then, 0.25 N Folin Ciocalteu, sodium carbonate 1 N, methanol and water were added to this extract. After 2 h of incubation at ambient temperature protected from light, the optical density was measured in a spectrophotometer at 725 nm. Data were presented as grams of gallic acid equivalents per kilograms of fruit (g/kg) in dry weight.

The total anthocyanins concentration on the samples was determined according to Zhang, Pang, Yang, Ji, Jiang (2004). To this purpose, 0.5 g of freeze-dried sample were extracted with 15 mL of acidified ethanol and incubated for 30 min at ambient temperature protected from light. The optical density was measured in a spectrophotometer at 535 nm. Data were presented as grams of pelargonidin equivalents per kilograms of fruit (g/kg) in dry weight.

The methodology described by Brand-Willians, Cuvelier & Berset (1959); Arnao, Canoa & Acosta (2001) was used to determine the total antioxidant activity. Thus, 0.5 g samples were extracted using 20 mL of methanol P.A. Then, 2.8 mL of DPPH (2,2-diphenyl-1-picryl-hydrazyl) and 0.17 mL of methanol were added to the extract. After 3 h of reaction at ambient temperature protected from light, the optical density was measured in a spectrophotometer at 515 nm. Data were presented as mM TE/g in DW.

All the analyses were performed in four biological replicates and three analytical replicates.

2.7. Characterization of individual phenolic compounds and L-ascorbic acid

The extraction of individual phenolic compounds was performed according to the method described by Hakkinen, Kärenlampi, Heinonen, Mykkanen & Torronen

(1998), with modifications. To this purpose, 0.5 g of lyophilized sample was dissolved in 30 mL methanol. After, 4.9 mL hydrochloric acid was added to the extract and homogenized for 24 h at 35 °C, in the dark. The mixture was filtered and the supernatant was concentrated in rotary evaporator at 40 °C for about 30 min. The residue was concentrated and re-dissolved in methanol to a final volume of 5 mL, which was centrifuged (7000 rpm for 10 min). The supernatant (30 µL) was injected into the chromatograph. We used a liquid chromatography (HPLC - Shimadzu), with auto sampler, UV-visible detector at 280 nm, phase column RP-18 reverse CLC-ODS (5 µm, 4.6 mm x 150 mm) with octadecylsilane stationary phase and a guard column CLC- GODS (4). The mobile phase gradient elution consisted of an aqueous acetic acid solution: methanol (99:1, v/v), with a flow rate of 0.8 mL min^{-1}, with a total run time of 45 min, following the methodology described by Zambiazi (1997). The results were expressed in g/kg of fruit in DW.

The content of L-ascorbic acid was also determined by the same high performance liquid chromatography, following the methodology described by Vinci, Rot & Mele (1995). The analyzes were performed on the following chromatographic conditions: 10 µL injection volume, flow rate of 0.8 mL min^{-1} with detection at 254 nm, and the mobile phase was a solution of 0.1 % acetic acid in ultrapure water and methanol 100 %. The L-ascorbic acid was used as the standard. The results were expressed in g L-ascorbic acid/kg fruit in DW.

The analyses were performed in four biological replicates and three analytical replicates.

2.8. Evaluation of relative expression of transcripts related with strawberry fruit quality

The expression of the following key transcripts of the phenylpropanoids pathway was evaluated: *PAL* and *UFGT*. We also evaluated the expression of transcripts from the ascorbate pathway: *GME* and *GLDH*.

The reference genes utilized to normalized the transcripts levels were *PIRUV_DESCARB* (encoding for piruvato descarboxylase), *UBQ11* (encoding for ubiquitin protein), and *HISTH4* (encoding for histone H4). These genes were selected according to previous evaluations performed at our laboratory (data not shown). For gene expression evaluation, total RNA of strawberry mature fruits was isolated using CTAB (hexadecyltrimethylammonium bromide), according Messias et al. (2014). RNA concentration was evaluated by the fluorometry technique (QuBit-RNA BR, Invitrogen). Total RNA (1 µg) was digested with 1U DNase I and DNase 1 × reaction buffer (Invitrogen) before cDNA synthesis. The digested RNA was reverse transcribed using the M-MLV enzyme and oligo-dT primers, according to manufacturer's instructions (Invitrogen). The sequences of the evaluated transcripts were extracted from GenBank database for the synthesis of specific primers, which were designed using Vector NTI10 software (Invitrogen), according to the following parameters: melting temperature (Tm) of 58-62 °C and GC content of 45-55%. All primers sequences are presented in Supporting Information Description 1. The cDNAs, in the concentration of 20 ng/µL were amplified by RT-qPCR in a final volume of 20 µL containing 1 µL cDNA, 10 µL of Platinum Sybr green UDG (Invitrogen), and 3-5 ρmol of each primer. Amplification was standardized in a 7500 Real time Fast thermocycler (Applied Biosystems) using the following conditions: 50 °C for 20 s, 95 °C for 10 min followed by 40 cycles of 15 s at 95 °C and 60 s at 60 °C.

The PCR products for each primer set were subjected to melt curve analysis in order to verify the presence of primer dimmers or nonspecific amplicons. The relative expression data was calculated according to the $2^{-\Delta\Delta Ct}$ method and presented as fold change (Livak & Schmittgen, 2001). The analyses were performed in four biological replicates and three analytical replicates.

2.9. Statistical analyzes

Statistical analyzes were performed using the computer program SAS System for Windows version 9.1.3 (SAS, 2000). Data obtained were analyzed for normality by the Shapiro-Wilk test, the homoscedasticity was assessed using the Hartley test and the independence of residuals was checked graphically. Data were subjected to variance analysis ($p \leq 0.05$). In case of statistical significance, we compared the fertilizers by the t test ($p \leq 0.05$). Data were recorded as mean ± standard deviation and relative expression of transcripts were recorded as mean ± standard error.

3. RESULTS AND DISCUSSION

3.1. The soil electrical conductivity was increased but the photosynthetic variables were not affected by comparing fertilizers

Among the factors affecting fruit production, fertilizer may be one of major effect (Ogendo, Isutsa & Sigunga, 2008). Soluble fertilizer are the most common source of nutrients currently used in agriculture; however, the negative effects on environment, the poorness of soil and the micronutrient deficiencies caused by their use has resulting in the search for alternative natural sources of fertilizers. Organomineral fertilizers can be important sources of many nutrients, which are

relevant to the development of plants and may result in higher productivity and fruit quality (Chassapis & Roulia, 2008). Therefore, we evaluated the effectiveness of using natural phosphate, granodiorite and tung press cake (AF), as natural sources of P, K and N, respectively, in the production of strawberry and compared with the currently used soluble/conventional fertilizer (SF).

Organomineral fertilizers such as the natural phosphate and granodiorite proposed in this study are commonly known to have low reactivity, and their nutrients are slowly released into the soil (Shivay et al. 2010; Silva et al. 2012).

Electrical conductivity is a measure that represents the phenomenon of transfer of electrons from charged particles, ionic solutes and colloids on an electric field, (Assiry, Gaily, Alsamee & Sarifudin, 2010). Therefore, this parameter may be used to evaluate the release dynamic of fertilizers in a soil. In fact, the analysis of electrical conductivity performed in the present study, throughout the culture cycle (Figure 2A), showed that in both fertilizers, the electrical conductivity of the soil increased significantly ($p \leq 0.05$) over time (from 76.13 to 452.63 uS/cm in SF and 36.95 to 531.34 uS/cm in AF). However, the electrical conductivity was statistically higher in SF than in AF up to the fifth measurement (150 days after transplanting, M5 in Figure 2), whereupon the electrical conductivity of SF decreased in the sixth measurement to lower values than AF (M6 in Figure 2). Thus, the supply of nutrients to the plant by both fertilizers increased along crop development, but at the end of the crop cycle, the AF was still able to provide increasingly amounts of nutrients, while the content of available minerals decreased in the SF treatment ($p < 0.05$). This result confirms the slower release rates of nutrients from the soil supplied with organomineral fertilizers, compared to soluble fertilizers. This result confirms the slower release rates of nutrients from the soil supplied with organomineral fertilizers,

compared to soluble fertilizers. This factor could be of great importance for annual crops and, in the case of strawberry, neutral days cultivars, that will require nutrients among a long time, in this cases, plants will benefit from a fertilizer that continues releasing nutrients. However, it is important to consider that the dynamics of nutrient release from these fertilizers should be accompanied for a longer period of time and on a second crop cycle to confirm these hypotheses largely.

Soil nutrient availability, as well as the chemical form which those nutrients are presented, greatly determine the balance of minerals that are absorbed by the plant (Fageria, Baligar & Jones, 2011; Jones, 2012). Consequently, the ability of plant to perform photosynthesis is influenced by the absorbed minerals, since they play an important role from CO_2 absorption to the conversion into carbohydrates (Ghaderi & Siosemartleh, 2011).

Therefore, in the present study, the values of CO_2 assimilation rate (A) (Figure 2B), transpiration rate (E) (Figure 2C) and total water vapor conductance (GH_2O) (Figure 2D) were evaluated in plants grown under the two fertilizers in order to assess whether the proposed AF affect the photosynthetic process. The results show that AF did not statistically affect the photosynthetic process when compared to SF ($p \leq 0.05$). In AF, A varied from 6.33 to 20.67 $\mu mol/m^2s$; E varied from 1.02 to 1.52 $mmol/m^2s$; and GH_2O from 43.79 to 185.07 $mmol/m^2s$. Mean while, these parameters ranged from 21.82 to 5.91 $mmol/m^2s$ (A), 1.45 to 0.98 $mmol/m^2s$ (E) and 175.80 to 45.46 $mmol/m^2s$ (GH_2O) in the SF treatment.

We also recorded the measurements of temperature and relative (Figure 2E) humidity in the greenhouse during the measurements of the photosynthetic variables. The temperature varied from 15.3 to 27.4°C and the relative humidity varied from 60.9 to 86.7 during the measurements. Therefore, the decrease in the value of the

photosynthetic variables over the crop cycle, as observed in Figure 2, may be a result of the increased temperature and decreased relative humidity, related to the seasons in the south of Brazil. Other studies showed that these variables vary considerably in strawberry plant, according to cultivar, climate and soil conditions (Keutgen, Noga & Pawelzik, 2005; Orsini, Alnayer, Bona, Maggio & Gianquinto, 2012).

3.2. The yield of fruits, as well as the fresh and dry biomasses of strawberry are affected by the fertilizers

Among the factors affecting fruit production, fertilizer may be one of major effect (Ogendo, Isutsa & Sigunga, 2008). Although it is assumed that the concentration of nutrients supplied to the plant in organomineral fertilizers at the early development is lower than that provided by a soluble fertilizer which is characterized by being readily available in the soil, the results show that this amount was sufficient for the strawberry plants nutrition. As shown in Figure 3A, up to the first sampling time (ST1) the two treatments showed similar yield values per plant. However, the yield obtained from the ST1 to the ST2 was significantly ($p < 0.05$) higher in the AF (191.85 g of fruit per plant) than in the SF treatment (153.9 g of fruit per plant). Consequently, the total yield (from the beginning to the end of the experiment) was higher in the AF (225.08 g of fruit/ plant) compared to the SF (171.53 g of fruit/ plant), which represent an improvement of 23.79 %. This increase in production may be explained by the slower release of nutrients from AF, providing nutrients in the last stage of crop cycle. Furthermore, several minerals, others than N, P and K, and organic compounds present in the constitution of the AF may have played a role for

plant nutrition, resulting in higher performance to convert them into accumulated macromolecules.

In contrary to the results observed for fruit yield, the fresh and dry biomass of plants (Figure 3B) cultivated with AF (51.34 ± 3.2 and 12.68 ± 1.41) was lower than plants cultivated with SF (61.93 ± 0.74 and 16.64 ± 0.70). These results suggest that there is a trade-off between yield and vegetative growth and the use of AF targeted the increase of yield ratio over the growth metabolism, which is of interest in the production of strawberry.

3.3. The mineral composition of strawberry fruits are affected by fertilizers

Despite that yield remains the aim of agronomic efforts, the nutritional quality of fruits is also important, since dietary deficiency of minerals is often related. Thus, the use of fertilization aiming to increase mineral content have become an important biofortification strategy (Gómez-Galera et al. 2010).

Therefore, the content of minerals in strawberry fruits cultivated with AF and SF was evaluated. As shown in Table 1, minerals that were found in significantly ($p \leq 0.05$) higher amounts in AF compared to SF treatments were Cu and B, while the content of Mn was significantly higher in SF than in AF. However, most of minerals were not significantly different between fertilizers.

3.4. Accumulation of some compounds with functional potential are affected by fertilizers

Studies toward biofortification of food with compounds with functional and nutritional potential have been focus of great interest due to the benefits provided to health. The strawberry has a significant content of compounds derived from the

phenylpropanoids pathway that have antioxidant activity and another compounds for example vitamins. Therefore, the content of total phenolic compounds and anthocyanins, L-ascorbic acid and total antioxidant activity were measured in strawberry fruit cultivated with both fertilizers.

The phenolic compounds (Figure 4A) and the total antioxidant activity (Figure 4D) did not differ in plants cultivated with AF or SF. The phenolic compounds content in the AF varied from 3.20 ± 0.13 to 3.66 ± 0.12 g/kg, and from 2.94 ± 0.13 to 3.82 ± 0.15 g/kg in the SF, in the two sampling times, respectively. The total antioxidant activity values varied from 2.95 ± 0.14 to 4.18 ± 0.10 mM TE/g fruit and from 2.60 ± 0.22 to 4.2 ± 0.25 mM TE/g fruit in the AF and SF respectively, between the sampling times.

However, the anthocyanins content (Figure 4B) and the L-ascorbic acid (Figure 4C) content were significantly higher in fruits from AF treatment compared to fruits from SF treatment in the first sampling time, representing an improvement in compounds with antioxidant potential that contribute to the quality of these fruits. The content of anthocyanins varied from 1.17 ± 0.04 to 1.36 ± 0.07 g/kg in AF and from 0.72 ± 0.05 to 1.35 ± 0.02 g/kg in the SF treatment, in both sampling times, respectively. This increase in the anthocyanins content in AF treatment was concurrent with the higher relative expression of the *PAL* gene (Figure 5E), which encodes for the first enzyme of the phenylpropanoid pathway, and the higher relative expression of *UFGT* gene (Figure 4F), which encodes for enzyme directly involved in the biosynthesis of anthocyanins (Basha, Sarma, Singh, Annapuma & Singh, 2006). Since anthocyanins are thought to be induced by light, one possible explanation for the increase in anthocyanins content in the AF treatment is that those plants showed

lower plant biomass, and thus the fruits were more exposed to sun light (Castañeda-Ovando, Pacheco-Ornández, Páez-Hernández, Rodríguez & Galán-Vidal, 2009).

The L-ascorbic acid content varied from 0.58 ± 0.03 to 0.99 ± 0.04 and from 0.55 ± 0.01 to 0.63 ± 0.06 g/kg in AF and SF (Figure 4C), respectively. Therefore, the L-ascorbic acid content was higher in AF treatment than SF treatment in the first sampling time, which was concurrent with the higher relative expression of the genes *GME* (first enzyme in the ascorbate pathway) and *GLDH* (directly involved with the synthesis of L-ascorbic acid).

The increase of anthocyanins and L-ascorbic acid content in the AF treatment, in the first sampling time, may be related with the more slowly release of nutrients in the alternative fertilizer due to the complex diversity and aggregation of this materials, which could generate a mild stress condition in the strawberry plants that improve the production of this defense compounds, which is corroborated with the electrical conductivity results found (Figure 2E). However, the results for the second sampling showed that do not had a negative effect in the yield and plant biomass with the another results, as electrical conductivity, yield and plant biomass (Figure 3).

The phenolic compounds composition of strawberry vary significantly with several factors, as genotype, agricultural practice, maturity and environment (Aaby, Mazur, Nes & Skrede, 2012). Since the accumulation of individual phenolic compounds is differentially regulated and the total content of phenolic compounds showed no statistical difference between fertilizations, we performed the characterization of the mainly phenolic compounds accumulated in fruits cultivated with both fertilizers.

As observed in Table 2, gallic acid is the majority phenolic compound in strawberry fruits of our experiment with the values between 7.99 to 15.27 g of gallic

acid kg of fruit for AF, and 8.25 to 14.60 g/kg for SF, follow by catechin, hydroxybenzoic acid, ellagic acid and p-coumaric acid (Table 4???). Fruits from the first sampling did not differ in the content of any phenolic compound. However, the content of gallic acid, hydroxybenzoic acid and ellagic acid were higher in fruits from AF than from SF, reaching values of 15.27 g/kg, 7.71 g/kg and 5.83 g/kg, respectively. The improvement of these compounds may be related to the higher availability of nutrients in the AF treatment compared to SF treatment in the end of crop cycle, providing an extra amount of nutrient that was used to induce the secondary metabolism without have negative effect on yield.

4. Conclusion

The results of this study showed that the use of the organomineral fertilizer (AF), using alternative sources of N, P and K is a promising strategy to improve yield and compounds with functional potential of strawberry fruits, most likely because it is slower release of nutrients and because of the presence of other minerals and organic compounds in its composition. However, further studies are necessary to optimize AF doses, aiming to reduce the amount of raw material, thus increasing the technical viability. Furthermore, the understanding of AF mechanisms of action might help to develop novel formulations that target a specific compound to be improved, such as the anthocyanin and L-ascorbic acid.

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FIGURE GRAPHICS

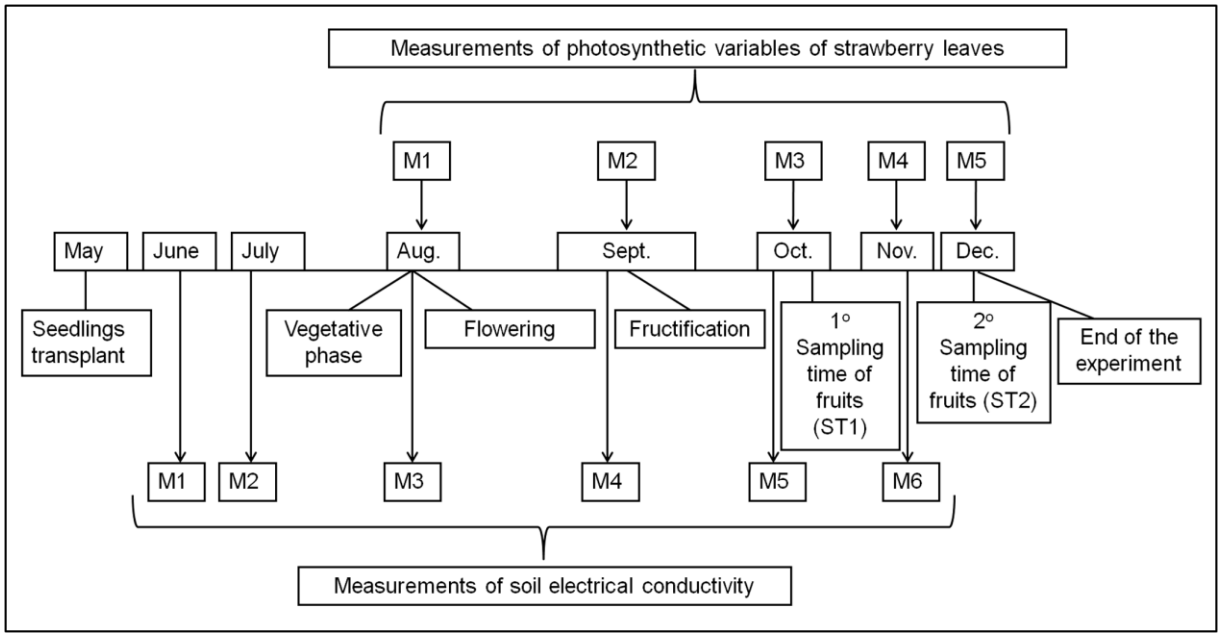

Fig 1. Timeline of the experiment, showing the crop cycle (transplanting, vegetative phase, flowering, fructification), sampling times, and closing of the experiment, as well as the measurements of photosynthetic parameters (M1 to M5) and soil electrical conductivity (M1 to M6), along the strawberry crop cycle.

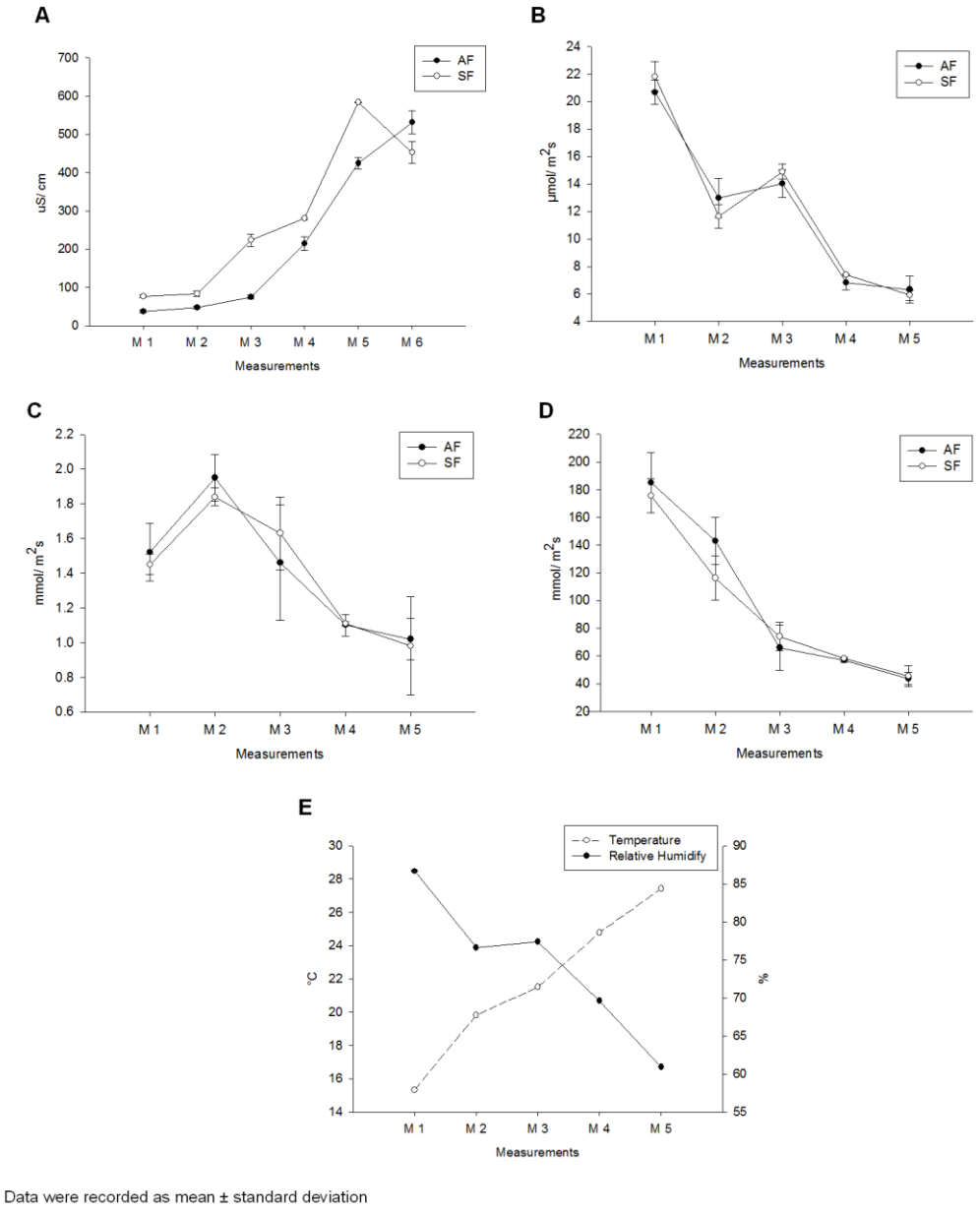

Fig 2. Soil Electrical Conductivity (µS/cm) (A) from the soil containing the Alternative Fertilizer (AF) and Soluble Fertilizer (SF) treatments used to cultivate strawberry plants. Photosynthetic Variables of strawberry leaves: assimilation rate - A (µmol/m^2s) (B), transpiration rate - E (mmol/m^2s) (C); water vapor conductance - GH_2O (mmol/ m^2s) (D); Temperature and Relative Humidity during the measurements (E). Measurements 1 to 6 (M1 to M6) are according to Figure 1 for photosynthetic variables and 1 to 5 for soil electrical conductivity.

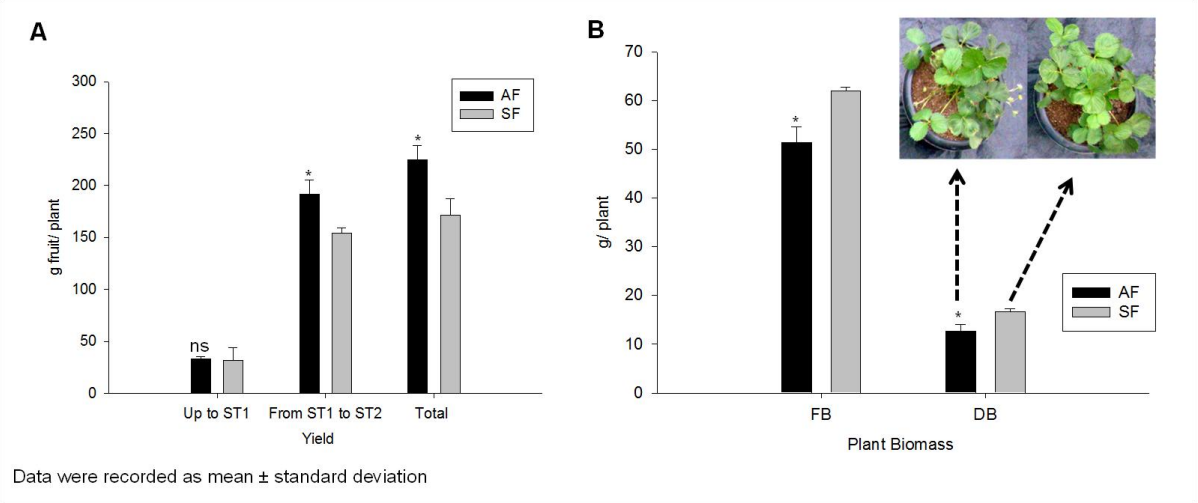

Fig 3. Yield of the strawberry fruits (A) and plant biomass (B) of the strawberry plants from the Alternative Fertilizer (AF) and Soluble Fertilizer (SF) treatments. The yield data was collected from the beginning of fruit production up to Sampling Time 1 (ST1); from ST1 to ST2; and from the beginning of fruit production to ST2 (Total yield). Fresh and Dry Plant Biomass (FB and DB) and their respective illustrations showed the difference between the plant biomass.

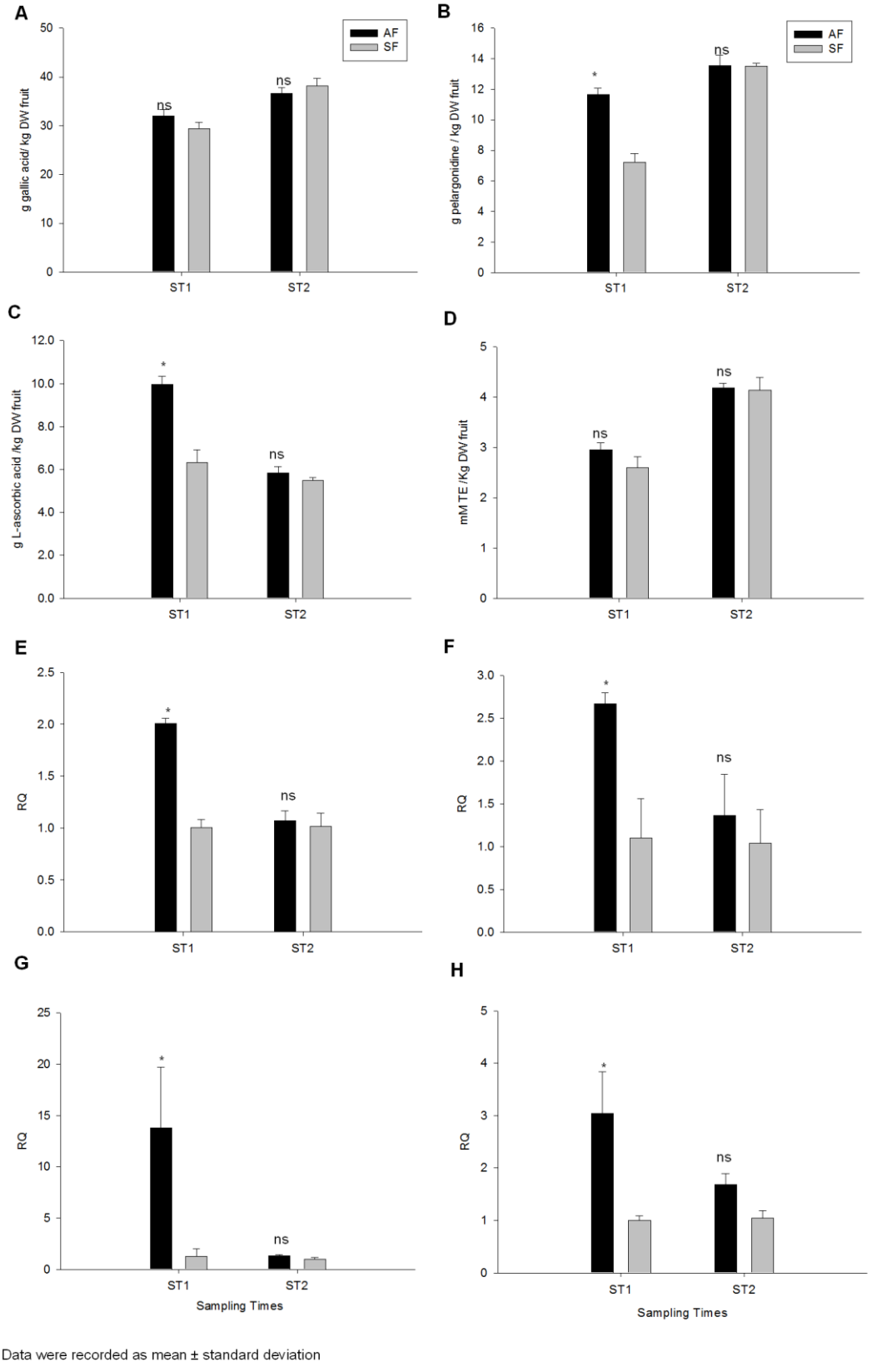

Fig 4. Compounds with potential functional in strawberry fruits: Total phenolic content (g gallic acid/kg DW fruit) (A); Total anthocyanins (g pelargonidin/kg DW fruit) (B); L-

ascorbic acid (g L-ascorbic acid/100kg DW fruit) (C); Total antioxidant activity (mM TE/kg) (D); Relative accumulation of transcripts of *PAL* gene (E); Relative accumulation of transcripts of *UFGT* gene (F); Relative expression of transcripts of *GME* gene (G); Relative expression of transcripts of *GLDH* gene (H) in strawberry fruits of Alternative Fertilizer (AF) and Soluble Fertilizer (SF) treatments from two sampling times (ST1 and ST2, according to Figure 1).

TABLES

Table 1

Mineral composition of strawberry fruits expressed in g of mineral per kg of fruit in dry weight (DW) (g/kg) of Alternative Fertilizer (AF) and SF in two sampling times.

Mineral	Sampling Times	Fertilizer	
		AF	SF
Ca	1	3.10 ± 0.36 ^{ns}	2.56 ± 0.61
	2	2.39 ± 0.29 ^{ns}	2.35 ± 0.11
Mg	1	2.59 ± 0.05 ^{ns}	2.34 ± 0.26
	2	3.15 ± 0.10 ^{ns}	3.29 ± 0.08
K	1	18.86 ± 0.88 ^{ns}	19.12 ± 0.82
	2	15.00 ± 0.62 ^{ns}	15.61 ± 1.35
P	1	2.63 ± 0.28 ^{ns}	2.61 ± 0.61
	2	1.81 ± 0.04 ^{ns}	1.82 ± 0.30
Cu	1	0.014 ± 0.008 *	0.007 ± 0.0004
	2	0.003 ± 0.0001 *	0.002 ± 0.0003
Mn	1	0.031 ± 0.0004 *	0.060 ± 0.001
	2	0.002 ± 0.002 *	0.059 ± 0.005
B	1	0.00043 ± 0.00002 ^{ns}	0.00044 ± 0.00009
	2	0.00017 ± 0.00002 *	0.00008 ± 0.00002

* and ^{ns} significative by t test ($p \leq 0,05$) and no significance respectively, comparing fertilizers

Table 2

Characterization of individual phenolic compound of strawberry fruits expressed in g of individual phenolic compound/kg of fruit in dry weight (DW) (g/kg) of Alternative Fertilizer (AF) and Soluble Fertilizer (SF) in two sampling times.

Individual Phenolic Compound	Sampling Times	Fertilizer	
		AF	SF
Gallic acid	1	7.99 ^{ns}	8.25
	2	15.27 *	14.60
Catechin	1	6.45 ^{ns}	6.00
	2	13.81 ^{ns}	13.55
Hydroxybenzoic acid	1	0.41 ^{ns}	0.31
	2	7.71 *	5.09
p-coumaric acid	1	0.25 ^{ns}	0.21
	2	0.42 ^{ns}	0.34
Ellagic acid	1	0.47 ^{ns}	0.39
	2	5.83 *	2.61

* and ^{ns} significative by t test ($p \leq 0,05$) and no significance respectively, comparing fertilizers

Supplementary data

Supplementary data 1

Source of nitrogen (N), phosphorus (P) and potassium (K) and their respective quantities of nutrients (kg/ha), of the Alternative Fertilizer (AF) and Soluble Fertilizer (SF), based on the recommendations for the SF (CQFS, 2004).

Recommendations of NPK[a]	Source of nutrients of the AF	Quantity of each compound of AF	Source of nutrients of the SF	Quantity of each compound of SF
260 kg/ha P_2O_5	Natural phosphate Bayovar	897	Triple Superphosphate	619
200 kg/ha K_2O	Granodiorite gnaissic	4651	Potassium Chloride	333
120 kg/ha N	Tung press cake	4800	Urea	267

[a]Defined according to the soil analysis and the crop recommendation. [b]The AF sources have other minerals nutrients in additional to NPK.

Supplementary data 2

Sequence of primers that were utilized for evaluate the gene expression.

Primer	*Forward*	*Reverse*	*Gen bank accession*
GLDH	TGGACAGGGAGAAGAAGCGA	AACAACTCCAAGACCGCCAA	According to Severo et al. 2011.
GME	CAACTGAGGAGTTGT	TCGGGTCTGAAGTCCATCTC	According to Cruz-Ruz et al. 2011
PAL[a]	CGAAAAACTGCAGAAGCAGTTGACA	TCAAGTTCTCCTCCAAATGCCTCAA	HM641823.1; AB360394.1; AB360393.1; AB360391.1; AB360390.1
UFGT	CAAGCAGTCCAACAGCTCAATC	GAAAACATACCCCTCCGGCAC	AY575056.1
PIRUV_DESCARB	AGGTGCGTTGCGAAGAGGA	CTAAATCTGTGAATGCGAATGAGG	AF141016.2
UBQ11	CAGACCAGCAGAGGCTTATCTT	TTCTGGATATTGTAGTCTGCTAGGG	According to Hytönen et al. 2009.
HISTH4	GTGGCGTCAAGCGTATCTCC	TGTCCTTCCCTGCCTCTTGA	AB197150.1

[a]*PAL* primers were designed based on a consensus sequence of these access.

4. Artigo 2 – “Effect of drought stress in photosyntetic variables, yield and antioxidant compounds in strawberry fruits”

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EFFECT OF DROUGHT STRESS IN PHOTOSYNTETIC VARIABLES, YIELD AND ANTIOXIDANT COMPOUNDS IN STRAWBERRY FRUITS

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Abstract

Background: Strawberry (Fragaria x ananassa) has a interest compounds for human health because these compounds has antioxidant activity, and their pleasant sensory characteristics. Strategies that is able to increase the content of potential nutritional and functional compounds in fruits, namely biofortification, have been widely studied, because of their health beneficial effects. In this context, in the present study, we evaluated the effect of levels of drought stress in a set of agronomic and biochemical responses related to fruit quality in order to verify the efficacy of drought stress as a biofortification effort. We also evaluated the relative expression of transcripts related with phenylpropanoids compounds.

Results: Photosynthetic variables, yield and plant biomass were affected by drought stress being reduced, mineral composition was has variance in Ca, Cu and B. Phenolic compounds, antioxidant activity and genes related with anthocyanins synthesis were increased in drought stress conditions.

Conclusion: The results indicates that the strategy of the drought stress for biofortifification in the strawberry crop have potential to increase compounds with antioxidant capacity of interest for the human health, but until it is necessary to meet a level that not affect fruit yield and plant development.

Keywords: strawberry, alternative fertilizer, drought stress, biofortification, yield, fruit quality

INTRODUCTION

The strawberry fruit is one of the most popular fruits because of its taste and the well-recognized health promoting properties; therefore, its demand and availability in the market has widely increased.[1] The strawberry (*Fragaria* × *annanasa*) belongs to the Rosaceae family, and it has several cultivars that differ in their sensory characteristics such as taste, size and texture. These cultivars also differ in the content of compounds derived from the phenylpropanoid metabolism and in the content of Vitamin C, which play a role in plants during stress conditions and they are considered potential functional compounds in human diet because of their antioxidant activity.[2,3] In south Brazil (Rio Grande do Sul state), the Camarosa cultivar is the most widely used strawberry cultivar because it is characterized by better agronomic performance, with good adaptation to different soil types and climates.[4]

Strategies that is able to increase the content of nutritional and functional compounds in fruits, namely biofortification, have been widely studied, because of their health beneficial effects, such as quenching of free radicals, combat of premature aging of cells, reduction of infections susceptibility, anti-inflammatory activity, among others. The main compounds in strawberry with this potential are: phenolic acids (p-coumaric and caffeic acid), flavonoids and anthocyanins (mainly cyanidin and pelargonidin). In plants, these compounds are synthesized in the phenylpropanoid pathway, where the phenylalanine ammonia lyase (*PAL*) is the entry enzyme, followed by a series of enzymatic reactions involving key enzymes such flavonoids UDP glycosyltransferase (*UFGT*), responsible for the synthesis of anthocyanins.[5] The biofortification efforts include several approaches, such as the

application of biostimulants or fertilizers, application of abiotic stresses, conventional breeding and genetic engineering.[6,7]

Currently, results of studies have indicated the possibility of increase the content of potential functional compounds through the application of abiotic stresses, without causing damage to the plant development.[8,9] These stresses most likely result in a metabolic signaling in the cell, leading to the increase in the content of antioxidant compounds and in the activity of antioxidant enzymes, as a defense mechanism;[10] consequently, there is an increase in the content of potential functional compounds in the fruit.

Drought stress occurs when there is a continuous loss of water or reduced water availability in the soil.[11] Therefore, the use of drought stress as a biofortification strategy may improve the quality of fruit and may also represent a sustainable strategy to reduce the water usage, an imminent necessity due to the current increase of population. However, the drought stress level necessary to induce those compounds and the mechanisms highlighting this effect are not well understood. In this context, in the present study, we evaluated the effect of levels of drought stress in a set of agronomic and biochemical responses related to fruit quality in order to verify the efficacy of drought stress as a biofortification effort. We also evaluated the relative accumulation of genes related with phenylpropanoids compounds to better understand the mechanisms involved in the stress response in strawberry.

MATERIALS AND METHODS

Plant material and experimental conditions

The experiment was conducted in a greenhouse at Brazilian Agricultural Research Corporation (Embrapa) (CPACT/Pelotas/RS/BR), between May to December 2012. Strawberry seedlings 'Camarosa' were transplanted and grown in pots of 9 L, containing a mixture of soil and vermiculite (3:1). The experimental design was completely randomized with four replications and three treatments. Treatments were arranged in one-factor scheme, as follow: C- control/normal irrigation, with 100 % of crop evapotranspiration (ETc); L1- drought stress level 1, with 70 % of crop ETc,; L2 - drought stress level 2, with 50 % of ETc. The irrigation was performed by dripping daily, and the water volume applied to irrigation was adjusted weekly, according to the ET value of the culture, as proposed by Marouelli et al [12]. The drought stresses (L1 and L2 treatments) were applied from the beginning of flowering stage (109 days after transplanting - DAT) to the end of crop cycle (212 DAT). The fertilizer was constituted by natural/ alternative sources of NPK. The quantity of each components of both fertilizers was calculated to obtain the equivalent of 267 kg ha^{-1} N, 619 kg ha^{-1} P_2O_5 and 333 kg ha^{-1} K_2O, according to the fertilizer recommendations for this crop and a preliminary analysis of the soil, based on the recommendations for the soluble fertilizer.[13] Two sampling times (ST1 and ST2) of mature fruits (stage eight according to Jia et al[14]) were performed during the crop productive stage. The fruits were weighed and immediately frozen with liquid nitrogen. The samples were storage in ultrafreezer at -80 °C and freeze dried to perform the fruit quality analysis. All analysis were performed in four field and three analytical replicates.

Evaluation of the effect of drought stress on agronomic variables and mineral composition of strawberry fruits

Photosynthetic variables

The CO_2 assimilation rate (A), total water vapor conductance (GH2O) and transpiration rate (E) of the plants were monitored with a portable gas exchange fluorescence system infrared gas analyzer (IRGA) (Heinz Walz GmbH, GFS 3000 model). Regular measurements were performed during the crop cycle in novel full-developed leaves in the vegetative phase until the end of the experiment, totalizing six measurements (M1 to M6). To determine the amounts of CO_2 and light to be used in the measurements, a standard curve with these variables was previously performed.

Soil electrical conductivity

The soil electrical conductivity was determined with a conductivimeter (Tecnal, TEC-4MPP model) in samples collected randomly from the pots, before (measurement M1 – correspondent of the seedlings transplant) and after (measurement M2 – correspondent to the end of the experiment) the treatment applications.

Yield of fruits and fresh and dry plant biomass

For determination of crop yield were collected and weighed during all the crop cycle, and the results were showed cumulative yield up to ST1, cumulative yield from ST1 to the ST2 and the total. Fresh and dry plant biomass were determined at the

end of the cycle by weighing the aerial portion of the plant, before and after drying in an oven with air circulation at 60 °C, respectively.

Mineral composition of strawberry fruits

The mineral composition was determined in lyophilized samples. The content of phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), copper (Cu), manganese (Mn) and boron (B) was quantified by atomic absorption spectrometry (AAS) (Varian™ AA240FS, Santa Clara, CA) from 250 mg of sample digested with 1 mL of H_2O_2 and 5 mL of HNO_3 in microwave, according to Silva[15]. Mean values were expressed as g of mineral per kg of fruit in dry weight (DW) (g kg DW $fruit^{-1}$). The analysis were performed in four field and three analytical replicates.

Evaluation of the effect of drought stress on the total phenolics, total anthocyanins, L-ascorbic acid and total antioxidant activity

Total phenolic content were quantified by the method from Swain, Hillis[16]. In summary, 0.5 g of freeze-dried sample were extracted with 20 mL of methanol P.A. Then, 0.25 N Folin Ciocalteu, 1 N sodium carbonate, methanol and water were added to the extract. After 2 h at ambient temperature protected from light, the optical density was measured in a spectrophotometer at 725 nm. Data were presented as grams of gallic acid equivalents per kg of DW fruit (g kg DW $fruit^{-1}$). The analysis were performed in four field and three analytical replicates.

The total anthocyanins concentration on the samples was determined according to Zhang et al[17]. To this purpose, 0.5 g of freeze-dried sample were extracted with 15 mL of acidified ethanol. After 30 min of reaction at ambient temperature protected from light, the optical density was measured in a

spectrophotometer at 535 nm. Data were presented as grams of pelargonidin equivalents per kilograms of DW fruit (g kg DW fruit^{-1}). The analysis were performed in four field and three analytical replicates.

The methodology described by Brand-Williams et al[18] and Arnao et al[19] was used to determine the total antioxidant activity. Thus, 0.5 g samples were extracted using 20 mL of methanol P.A. To this extract were added 2.8 mL of DPPH (2,2-diphenyl-1-picryl-hydrazyl) and 0.17 mL of methanol. After 3 h of reaction at ambient temperature protected from light, the optical density was measured in a spectrophotometer at 515 nm. Data were presented as mM of trolox per kilograms of DW fruit (mM kg DW fruit^{-1}). The analysis were performed in four field and three analytical replicates.

The content of L-ascorbic acid in fruits of strawberry was determined by high performance liquid chromatography (HPLC), following the methodology described by Vinci et al[20]. The liquid chromatograph used was a Shimadzu LC-10AT equipped with UV detector system in a reverse phase column (RP-18, 5 µm, 4.6 x 250 mm). The analyzes were performed on the following chromatographic conditions: 10 µL injection volume, flow rate of 0.8 mL min^{-1} with detection at 254 nm, and the mobile phase was a solution of 0.1 % acetic acid in ultrapure water and methanol 100 %. The L-ascorbic acid was used as a standard. The results were expressed in grams of L-ascorbic acid per kilogram of DW fruit (g L-ascorbic acid kg DW fruit^{-1}).

Enzymatic activity of Phenylalanine ammonia lyase (PAL) peroxidase (POD) and polyphenol oxidase (PPO)

For the determination of PAL activity, we followed method described by Hyodo, Yang[21] Hyodo et al[22] with modifications of Campos et al[23]. The extract was

obtained by adding borate buffer pH 8.5 to the samples and after washing and centrifugation steps. Sixty microliters of phenylalanine was added to the extract, followed by 1 h at 45 °C. The optical density was measured in a spectrophotometer at 290 nm. The same procedure was carried out with the same amount of water as negative control. The results were expressed in µmoL of transcinamic acid h^{-1} g of fruit.

To verify the oxidative status of the plants were determined the activity of peroxidase (PO, EC 1.11.1) and polyphenol oxidase (PPO, EC 1.14.18.1), according to the method of Campos et al[24]. Ideal conditions of pH (phosphate buffer pH 7.0) were provided and samples were centrifuged to obtain the extract for both enzymes. For the determination of PPO activity, we added 3.6 mL of the phosphate buffer, 1 mL of the extract and 0.1 mL of catechol 0.1 M, and 0.2 mL of 1.4 % perchloric acid. After 10 min. the optical density was measured in a spectrophotometer at 395 nm. For the determination of the PO activity, we added 2.5 mL of phosphate-citrate buffer, 1.5 mL of extract, 0.25 mL of guaiacol 0.5 %, and 0:25 mL of hydrogen peroxide 3 %. Samples were then incubated at 30°C in a water bath for 15 min. After this time, we added more 0.25 mL of sodium meta bisulphite at 2 % and incubated for 10 min. The optical density was measured in a spectrophotometer at 450 nm.

Evaluation of relative expression of transcripts related with the phenylpropanoids pathway

The expression of the following key transcript of the phenylpropanoids pathway was evaluated: *PAL* (encodes for phenylalanine ammonia lyase) *UFGT* (encodes for UDP flavonoid glycosyl transferase, responsible for the production of flavonoids and anthocyanins). And key transcript of the ascorbate pathway: *GME*

(encoding for GDP-D-mannose-3, 5-epimerase) *GLDH* (encoding for - L-galactono-1,4-lactone dehydrogenase responsible for the synthesis of ascorbate).

The reference genes utilized to normalized the transcripts levels were *PIRUV_DESCARB* (encoding for piruvato descarboxylase), *UBQ11* (encoding for ubiquitin protein), and *HISTH4* (encoding for histone H4). These genes were selected according to previous evaluations performed in our laboratory (data not shown). For gene expression evaluation, total RNA of strawberry mature fruits was isolated using CTAB (hexadecyltrimethylammonium bromide), according Messias et al[25] RNA concentration was evaluated by the fluorometry technique (QuBit-RNA BR, Invitrogen). Total RNA (1 µg) was digested with 1U DNase I and DNase 1 × reaction buffer (Invitrogen) before cDNA synthesis. The digested RNA was reverse transcribed using the M-MLV enzyme and oligo-dT primers, according to manufacturer's instructions (Invitrogen). The sequences of the evaluated genes were extracted from GenBank database for the synthesis of specific primers, which were designed using Vector NTI10 software (Invitrogen), according to the following parameters: melting temperature (Tm) of 58-62 °C and GC content of 45-55 %. The cDNAs, in the concentration of 20 ng μL^{-1}, were amplified by RT-qPCR in a final volume of 20 µL containing 1 µL cDNA, 10 µL of Platinum Sybr green UDG (Invitrogen), and 3-5 ρmol of each primer. Amplification was standardized in a 7500 Real time Fast thermocycler (Applied Biosystems) using the following conditions: 50 °C for 20 s, 95 °C for 10 min followed by 40 cycles of 15 s at 95 °C and 60 s at 60 °C. The PCR products for each primer set were subjected to melt curve analysis in order to verify the presence of primer dimmers or nonspecific amplicons. The relative expression data was calculated according to the $2^{-\Delta\Delta Ct}$ method and presented as fold change.[26]

Statistical analyzes

Statistical analyzes were performed using the computer program SAS System for Windows version 9.1.3.[27] Data obtained were analyzed for normality by the Shapiro-Wilk test, the homoscedasticity was assessed using the Hartley test and independence of residuals was checked graphically. Data were subjected to variance analysis ($p \leq 0.05$). In case of statistical significance, we compared the treatments by tukey ($p \leq 0.05$). Data were recorded as mean ± standard deviation and relative expression of transcripts were recorded as mean ± standard error.

RESULTS AND DISCUSSION

Evaluation of the effect of drought stress levels on agronomic variables and mineral composition of strawberry fruits

Strawberry crop is very sensitive to water deficit; therefore, water intake by strawberry plants may determine their agronomic performance and the quality of fruits.[1] Since an efficient photosynthesis and nutrient uptake from the soil are fundamental to obtain an adequate production,[28] we evaluated these variables in plants subjected to drought stress. As show in figure 1, the strawberry plants from the drought stress treatments (L1 and L2) showed a significant reduction in the photosynthetic variables, including CO_2 assimilation rate (**A**), transpiration rate (**B**), and water vapor conductance (**C**). We also observed a reduction in these variables in all treatments along the crop cycle, because of the increase in the environmental temperature along the experiment, which was compensated by an increase in the

day light, since all measurements were performed at the same hour (from 11-14 a.m.) of the day, in leaves from the same developmental stage.

During the crop cycle, the CO_2 assimilation rate varied from 20.67 to 6.33 µmol m^{-2} s^{-1} in the control plants; from 20.44 to 4.62 µmol m^{-2} s^{-1} in plants subjected to the low level of drought stress (L1); and from 18.09 to 2.12 µmol m^{-2} s^{-1} in plants subjected to the high level of drought stress (L2). Moreover, the transpiration rate varied from 1.52 to 1.02, 1.49 to 0.53, and 1.49 to 1.02 mmol m^{-2} s^{-1} in plants from the control, L1 and L2 treatments, respectively. A variation of 185.07 to 34.07 mmol m^{-2} s^{-1} in the control plants, 181.18 to 28.17 mmol m^{-2} s^{-1} in plants from L1, and 177.57 to 9.3 mmol m^{-2} s^{-1} in plants from L2 was observed in the water vapor conductance. Other studies have also demonstrated the influence of drought stress on the photosynthetic performance of strawberry plants,[29-33] confirming the results obtained in the present study.

Another parameter to monitor the drought stress is the quantification of soil electrical conductivity, which inform about the electricity exchange capacity between the ions from the soil,[34] and allow us to predict the movement of minerals from soil to the plant. Therefore, we evaluated the soil electrical conductivity before and after the application of the stresses, in order to verify the influence of water as a vehicle for minerals transport into the root. As show in Fig. 1D, the soil electrical conductivity t before the application of the stresses was similar in all treatments (around 37 µS cm^{-1}), confirming that they received the same soil. However, after the application of the drought stresses, the soil electrical conductivity was statistical higher in the L2 treatment (244.33 µS cm^{-1}) than in the L1 and control treatments (200.75 and 189.73 µS cm^{-1}, respectively). This result indicates that there is a higher content of ionic solutes in the soil of L2 treatment, because the reduction of the water availability

hampered the absorption of those solutes by the plant. Consequently, plants from the L1 and L2 treatments showed lower values of yield and biomass, compared to the control (Table 1).

This reduction in plant biomass may also limited the photosynthetic capacity of leaves, resulting in lower fruit yield,[35] in addition to obtaining fruits with reduced sizes, as observed in Table 1.

The evaluation of the mineral composition (Table 2) reveled that Ca, Cu and B were significantly reduced in strawberry fruits subjected to the drought stresses. Moreover, the content of Cu was also reduced in the leaves of those plants. These results may explain the lower photosynthetic efficiency and biomass production in the drought stressed plants. Ca is involved in a range of functions in cells, acting in various regulatory processes,[36,37] and Cu and B act on the photosynthetic apparatus and as cofactor of various enzymes.[38] Cu also acts as an element of the redox active transition, primarily operating in photosynthesis, respiration and enzymatic reactions, as well as in the metabolism of carbohydrates, nitrogen and lignin.[38] B also acts in the ion flux in the membranes, in the nitrogen metabolism, in the accumulation of polyamines and phenolic compounds, among others.[39-41] Contrarily, Mn and K were significantly increased in the leaves of drought stressed plants. K acts on different processes and is closely related to the yield and quality of crops and also assists in maintaining turgor and acts as a cofactor of several enzymes.[42] Furthermore, Mn acts on the photosynthetic apparatus, presenting significant role as a cofactor of various enzymes.[43]

Overall, it is clear that the photosynthetic capacity and production of fruits and leaves are strongly influenced by the level of water supplied to the plants, and by the levels of mineral nutrients.

Effect in the antioxidants compounds and relative expression of transcripts related to the phenylpropanoid pathway in strawberry fruits subjected to drought stresses

In addition to the agronomic variables, drought stress may also influence the accumulation of antioxidant compounds, since the plants are able to modulating defense responses, synthesizing specific compounds under stress conditions.[44,45] Among these compounds, the polyphenols are broken down into a range of classes of compounds, most of them exhibiting antioxidant activity.[46] Therefore, several studies have used the strategy of application of abiotic stresses as a feasible biofortification effort, because it increases the content of potential functional compounds, and provide greater resistance to subsequent stressful situations (increased tolerance).[47]

Thus, we evaluated the content of phenolics, anthocyanin, L-ascorbic acid and total antioxidant activity in fruits subjected to stresses and compared with the relative expression of transcripts and enzymes related to these compounds. As shown in Fig 3, the content of phenolic compounds, anthocyanins and L-ascorbic acid, as well as the antioxidant activity were influenced by the drought stress levels. More specifically, the content of phenolic compounds and the antioxidant activity were higher in plants exposed to drought stress, compared to the control, as observed in the second sampling time (Fig. 3 A/D). The same behavior was observed regarding the enzymatic activity of PAL and relative expression of PAL transcript (Fig. 5A), confirming this results. The PAL enzyme participates in the reaction of amino acid deamination of L-phenylalanine to form cinnamic acid, a precursor of the metabolic pathway of phenylpropanoids.[48]

Increased PAL enzyme activity and relative expression was also accompanied by increased activity of enzymes and oxidants PO PFO, associated with the increase in stress level. It is remarkable that these enzymes are critical to obtaining quality fruits.

Peroxidases are involved in various metabolic processes in plants, such as bridging between the components of the cell wall and the oxidation of cinnamyl alcohols prior to its polymerization during the formation of lignin and suberin, promoting the oxidative coupling by means of a monolignols dependent reaction of free radicals from hydrogen peroxide in the last stage of the biosynthesis of lignin, thus generated coloring products often in summary participate in oxidation of peroxides. Already the polyphenol oxidases act on phenolic compounds, promoting oxidation and polymerization, also leading to browning[49,50] and are produced in oxidative stress conditions, due to the imbalance between free radicals and compounds antioxidant activity.[51]

The anthocyanins and L-ascorbic acid were impaired in conditions of water stress exposed, because they suffered reductions. The total anthocyanins were higher and significant in control fruits (12.02 g kg DW fruit^{-1}) in the first sampling, when compared to the level 1 and 2, which showed statistically similar values among them: 9.96 g kg DW fruit^{-1} and 9.59 g kg DW fruit^{-1} respectively (Figure 3B).

But in the second sampling time, the control fruits and those belonging to level 2 was significantly greater than level 1. Corroborating these results, the relative expression of *UFGT* transcript in the level 2 at first sampling and in the two levels of drought stress in the second sampling (Figure 5B) showed decreasing.

In parallel, the L-ascorbic acid content of fruits showed the following behavior (Figure 3C): the first sampling, the control fruits showed higher levels in g L-ascorbic

acid kg^{-1} (9.95) than the level 1 and 2 respectively (7.70 and 9.11). While in the second sampling, only the content of L-ascorbic acid of level 2 of the drought stress fruits was affected (3.79) when compared to control fruits (5.85) and level 1 (5.74). This decreased were accompanied with the decrease in the relative expression of *GME* and *GLDH* transcripts that encoding for GDP-D-mannose-3, 5-epimerase and galactono-1,4-lactone dehydrogenase responsible for the synthesis of ascorbate, respectively.

.

CONCLUSION

The results indicates the viability of the drought stress as strategy for biofortification in the strawberry crop showed potential to increase compounds with antioxidant capacity of interest for the human health. In the level 1 of drought stress the results showed a increase in the photosynthetic variabless and in the fruit productivity related to the more severe level of stress (level 2). Although that remains necessary the search for a drought stress level that besides increasing compounds with antioxidant potential, as observed in this study, do not affect significantly the plant growth and yield of strawberry crop to be used as an effective biofortification technique.

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Tables

Table 1. Yield of fruits and fresh and dry plant biomass of strawberry plants

Treatments	Yield	Biomass	
		Fresh	**Dry**
Control	226.48 ± 9.91 a	51.34 ± 3.62 a	12.68 ± 1.82 a
L1	152.95 ± 3.52 b	35.44 ± 2.42 b	8.96 ± 1.09 b
L2	111.77 ± 2.20 c	21.89 ± 2.13 c	6.79 ± 0.91 c

[1/ns] Average followed by different letter in the same column comparing treatments, differ among themselves by tukey test ($p \leq 0.05$) and no significance, respectively.

Table 2. Mineral composition of strawberry fruits (g kg^{-1})

Mineral	Collect	Control	Level 1	Level 2
Ca	1	3.22 ± 0.36 a	1.45 ± 0.15 b	2.12 ± 0.37 b
	2	2.39 ± 0.29 ba	2.62 ± 0.09 a	2.02 ± 0.08 b
	Leaves	11.27 ± 0.47 ^{ns}	12.09 ± 0.08	11.81 ± 0.43
K	1	17.39 ± 1.36 ^{ns}	18.71 ± 1.70	17.29 ± 0.71
	2	14.99 ± 0.62 ^{ns}	14.36 ± 0.34	13.61 ± 2.77
	Leaves	15.96 ± 3.26 b	19.86 ± 1.75 a	19.13 ± 1.83 a
Mg	1	2.73 ± 0.05 ^{ns}	2.45 ± 0.32	2.62 ± 0.30
	2	3.13 ± 0.10 ^{ns}	3.29 ± 0.18	3.06 ± 0.68
	Leaves	9.47 ± 0.17 ^{ns}	9.18 ± 0.35	9.08 ± 0.32
P	1	2.40 ± 0.25 ^{ns}	2.71 ± 0.07	2.43 ± 0.20
	2	1.66 ± 0.07 ^{ns}	1.68 ± 0.09	1.55 ± 0.03
	Leaves	3.41 ± 0.24 ^{ns}	3.46 ± 0.34	3.37 ± 0.28
Mn	1	0.0314 ± 0.00043 ^{ns}	0.0270 ± 0.028	0.0255 ± 0.033
	2	0.0204 ± 0.0018 ^{ns}	0.0272 ± 0.032	0.0245 ± 0.11
	Leaves	0.087 ± 0.011 b	0.114 ± 0.016 a	0.115 ± 0.026 a
Cu	1	0.014 ± 0.002 a	0.006 ± 0.0002 b	0.004 ± 0.0002 b
	2	0.003 ± 0.0001 ^{ns}	0.003 ± 0.0002	0.003 ± 0.0006
	Leaves	0.024 ± 0.003 a	0.009 ± 0.0003 b	0.005 ± 0.0007 c
B	1	0.00042 ± 0.00002 ^{ns}	0.00047 ± 0.00007	0.00049 ± 0.00008
	2	0.00016 ± 0.00001 a	0.00006 ± 0.00001 b	0.00006 ± 0.00001 b
	Leaves	0.00057 ± 0.00002 ^{ns}	0.00057 ± 0.00001	0.00067 ± 0.00009

[1]/^{ns} Mean ± standard deviation. Different letter in the same line indicates significant difference among treatments from the same sampling time, according to the Tukey test ($p \leq 0,05$).

Illustrations

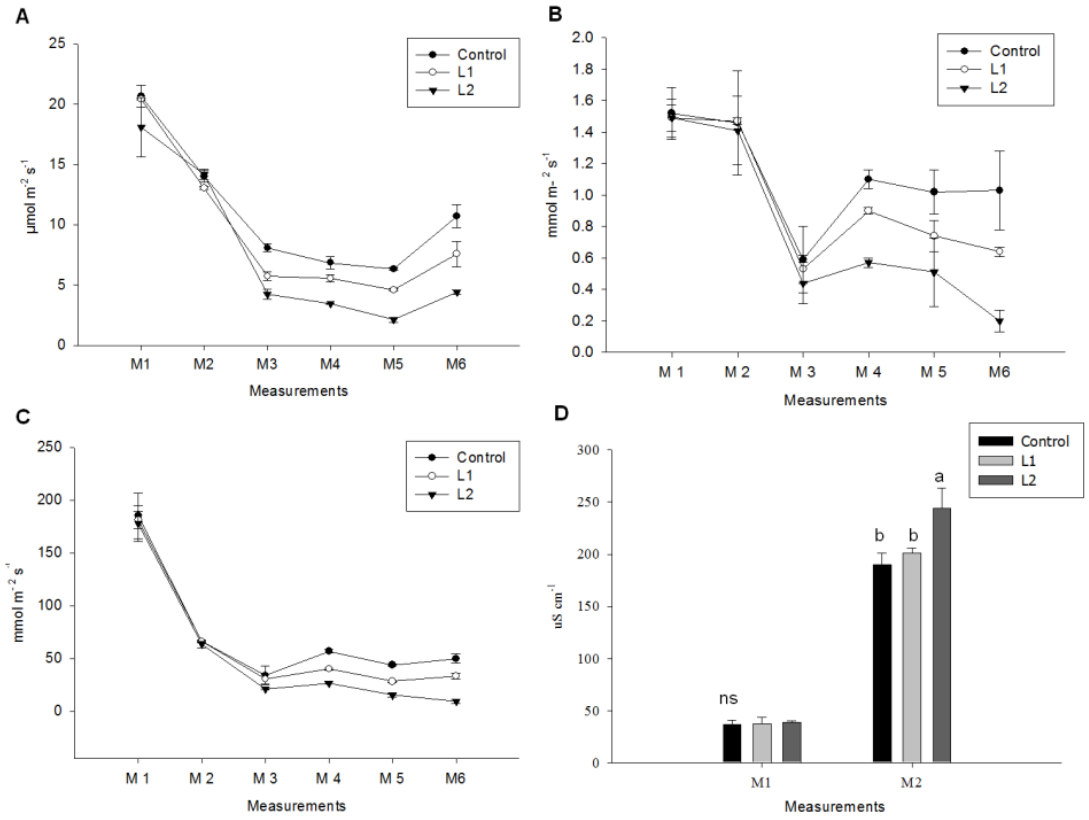

Figure 1. Photosynthetic Variables: assimilation rate (A) **(A)**, transpiration rate (E) (**B**), water vapor conductance (GH2O) **(C)** and Soil Electrical Conductivity **(D)** of strawberry plants. Measurements 1 to 6 performed during the crop cycle in novel full-developed leaves in the vegetative phase until the end of the experiment (M1 to M6) for A,B and C, and M1 and M2 correspondent of the seedlings transplant and end of the experiment respectively for soil electrical conductivity.

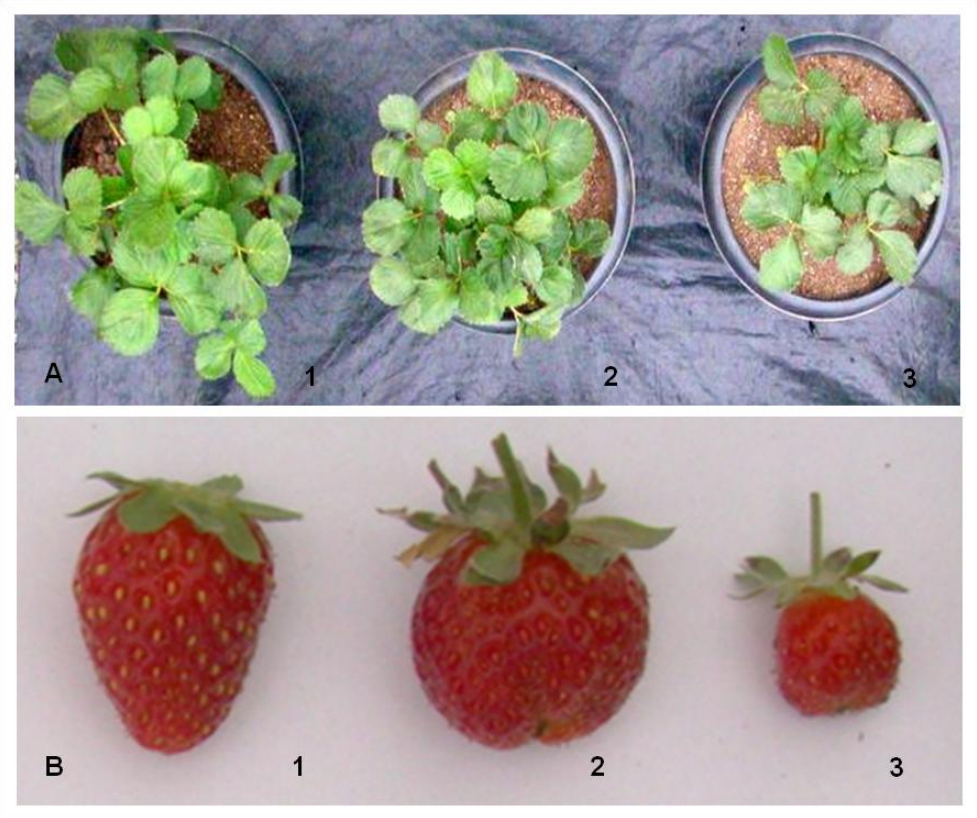

Figure 2. Strawberry plants and fruits submitted to drought stress. **(A)** Plants submitted to drought stress: control **(A1)**, Drought stress level 1 **(A2)** and drought stress level 2 **(A3)**. **(B)** Strawberry fruits correspondent of the plants submitted to drought stress: control **(B1)**, Drought stress level 1 **(B2)** and drought stress level 2 **(B3)**.

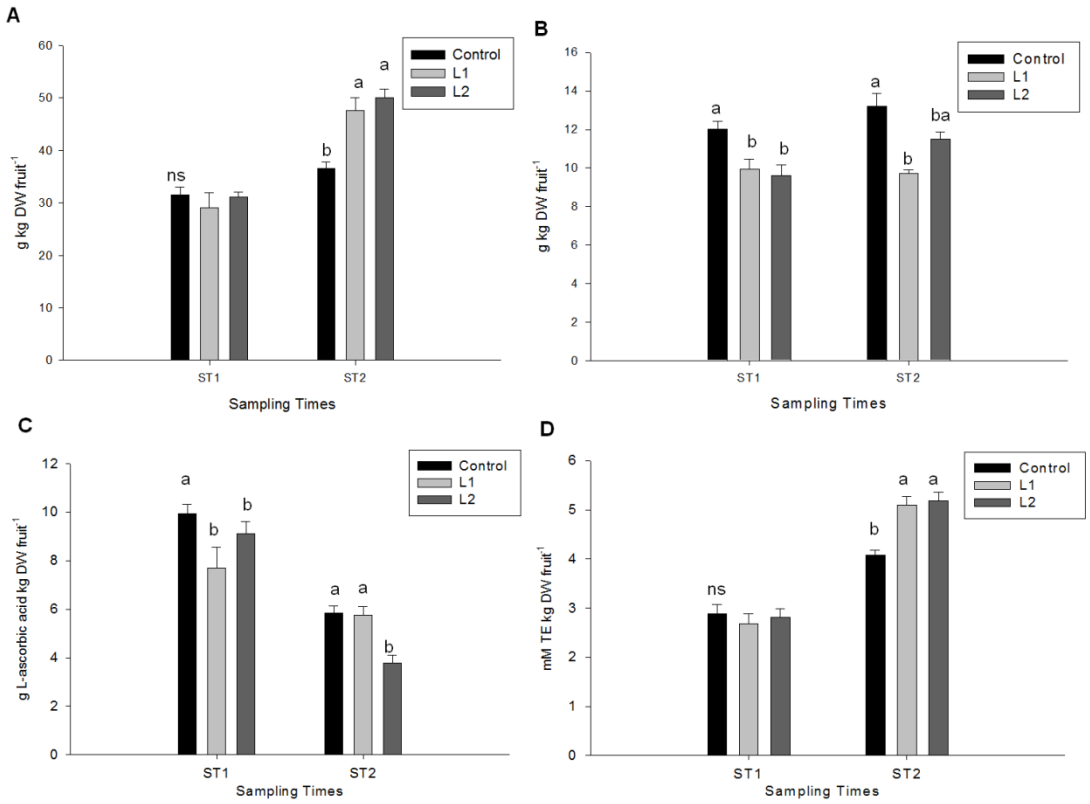

Figure 3. Quantification of: total phenolic content (g kg DW fruit^{-1}) **(A)**; total anthocyanins (g kg DW fruit^{-1}) **(B)**, L-ascorbic acid (g kg DW fruit^{-1}) **(C)**; total antioxidant activity (mM TE g^{-1}) **(D)** of strawberry fruits submitted to drought stress from two sampling times (ST1 and ST2).

[1/ns] Mean ± standard deviation. Different letter indicates significant difference among treatments from the same sampling, according to the Tukey test ($p \leq 0.05$).

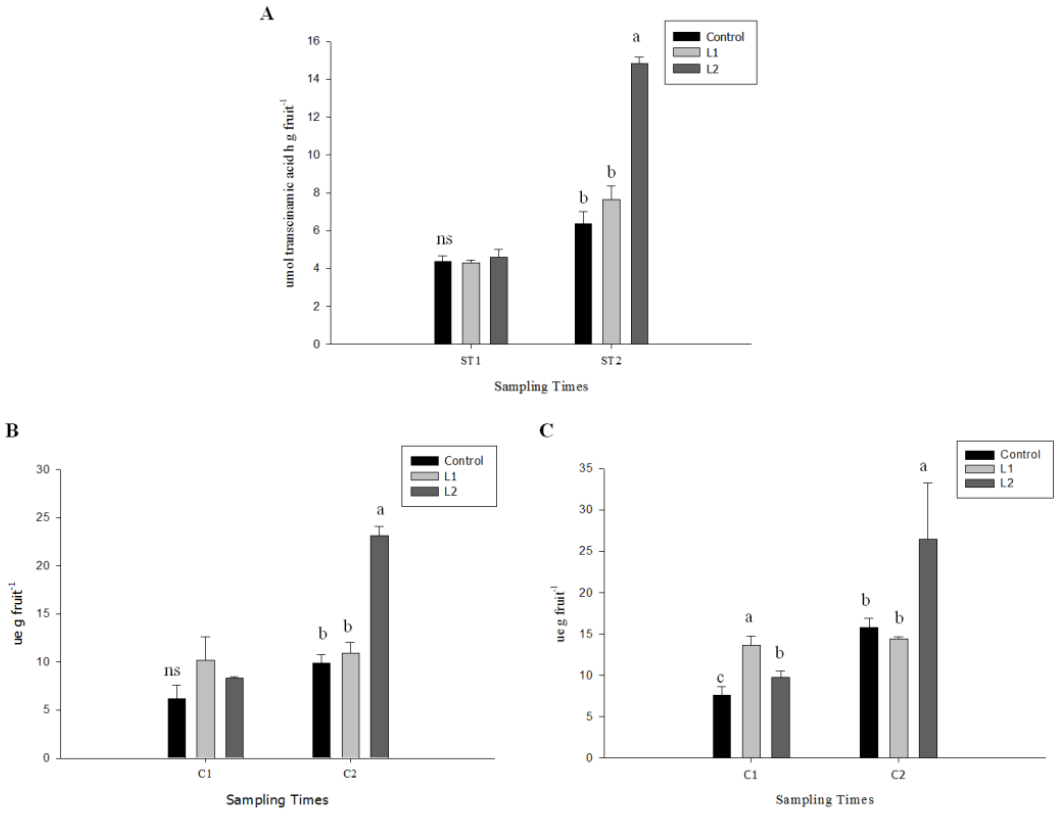

Figure 4. Activity of the enzhymes: Phenylalanine ammonia lyase (PAL) **(A)**; oxidative enzyme peroxidase (POD) **(B)** and polyphenol oxidase (PPO) **(C)**, of strawberry fruits submitted to drought stress from two sampling times (ST1 and ST2).

[1]/ns Mean ± standard deviation. Different letter indicates significant difference among treatments from the same sampling, according to the Tukey test ($p \leq 0.05$).

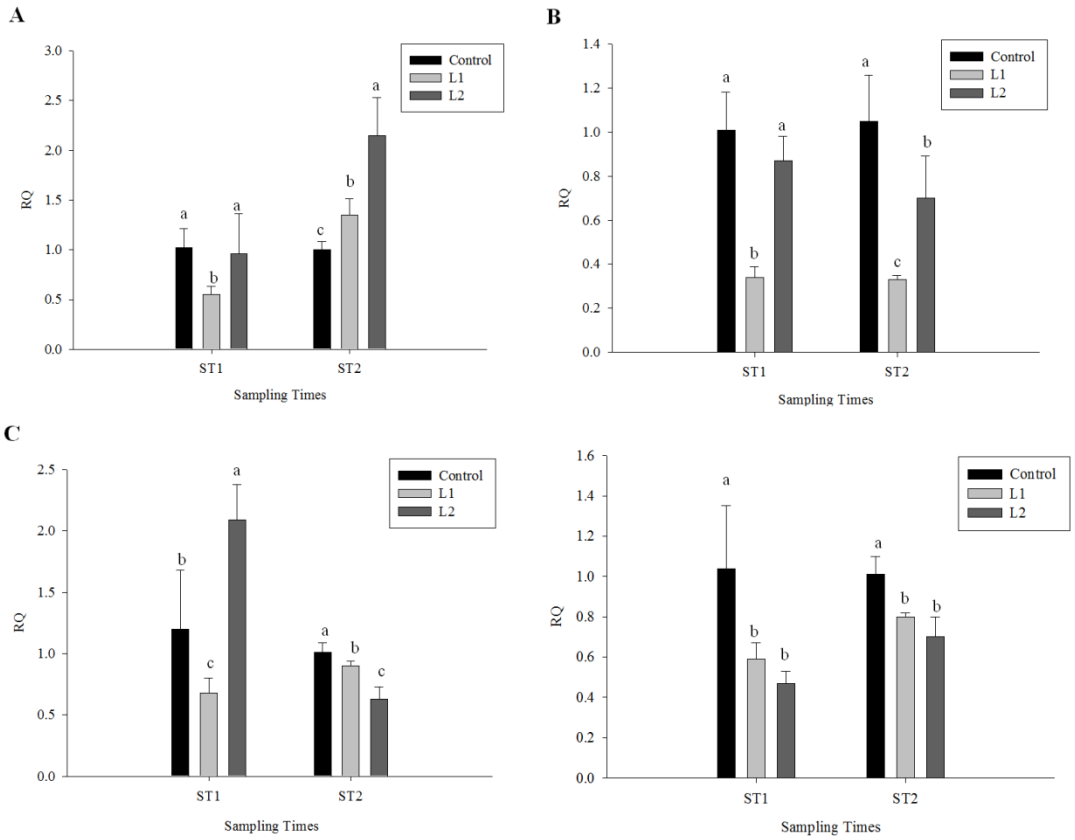

Figure 5. Expression relative levels of *PAL* **(A)**; *UFGT* **(B)**; *GME* **(C)**, *GLDH* **(D)** of strawberry fruits submitted to drought stress from two sampling times. The reference sample is the control treatment. [1/ns] Mean ± standard error. Different letter indicates significant difference among treatments from the same sampling, according to the Tukey test ($p \leq 0.05$).

5. CONSIDERAÇÕES FINAIS

Nas condições em que foram empregadas, este estudo permitiu verificar que a adubação baseada em fontes minerais e orgânicas de liberação gradual de nutrientes apresentou vantagens significativas na sua utilização, quanto à produtividade de frutos, sem no entanto afetar a capacidade fotossintética, além de um incremento em alguns compostos com potencial antioxidante, como antocianinas e ácido L-ascórbico, e estímulo de alguns compostos fenólicos e minerais, sendo portanto um adubação com potencial de uso na cultura do morangueiro. Além disso, essa adubação aliada a diferentes níveis de estresse hídrico apresentou um incremento nos compostos fenólicos e atividade antioxidante, muito embora tenha afetado as variáveis agronômicas e reduzido à produção dos frutos. Contudo, ainda se faz necessário buscar um nível de estresse que além de incrementar compostos com potencial antioxidante, não afete significativamente a produtividade da cultura e o desenvolvimento das plantas, para que possa ser utilizada como uma técnica eficaz de biofortificação na cultura do morangueiro.

Importante destacar que em estudos futuros, seja dada especial atenção às condições experimentais, principalmente no que diz respeito ao controle da variação da temperatura e da luminosidade, visto a grande magnitude de variação do primeiro e a interferência direta do segundo sobre a cultura do morangueiro.

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Apêndice

Apêndice A - Mudas de morango ‘Camarosa’ utilizadas no experimento.

Apêndice B - Preparo e enchimento dos vasos a partir da mistura de solo, vermiculita e respectiva adubação.

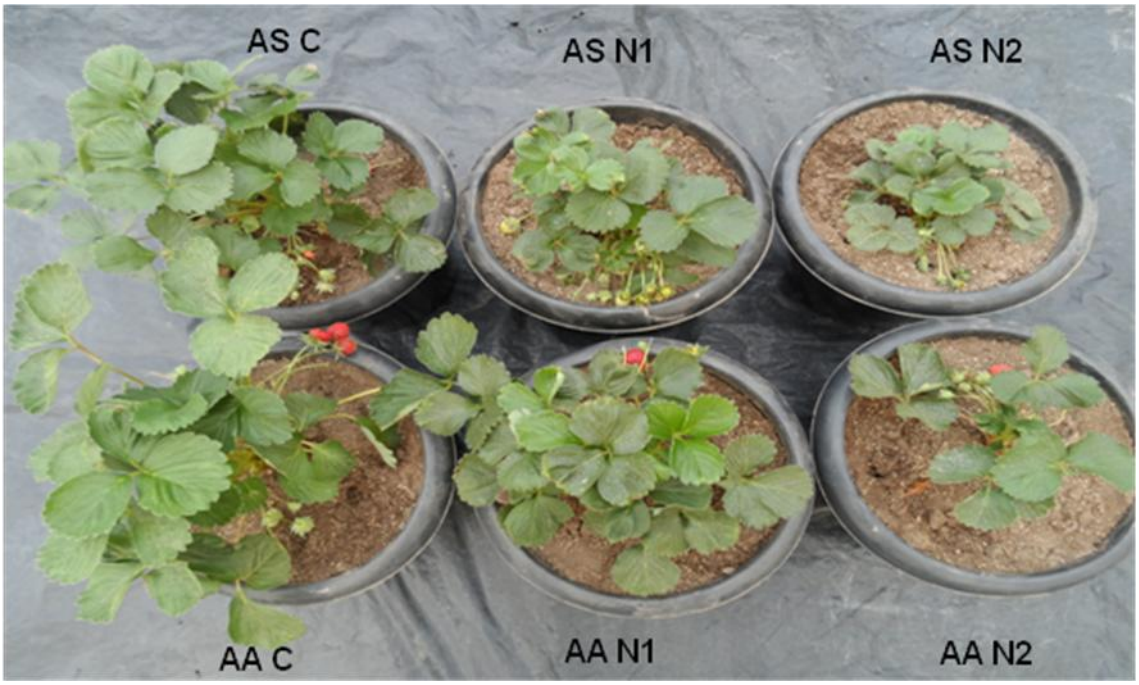
Apêndice C - Transplante das mudas para o desenvolvimento do experimento.

Apêndice D - Visão geral do experimento na fase vegetativa dos morangueiros.

Apêndice E - Prática de polinização manual dos morangueiros a partir da fase de florescimento.

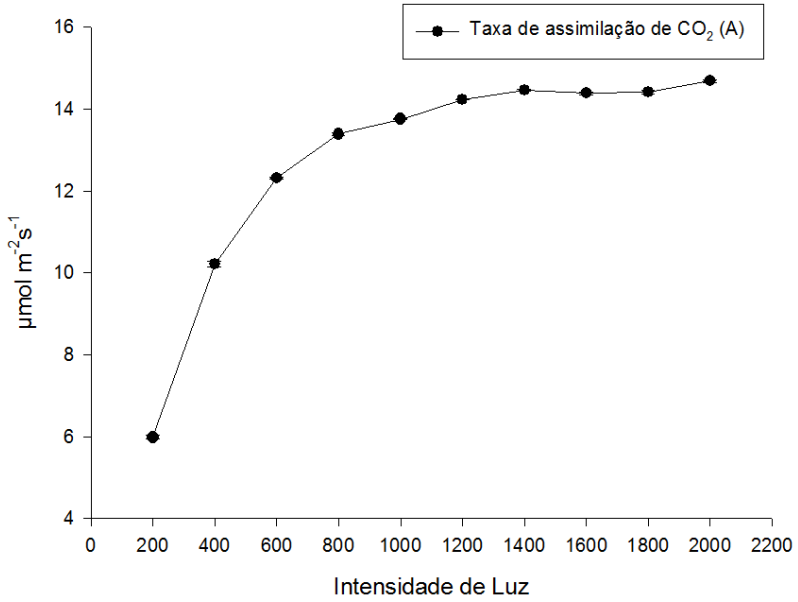
Apêndice F - Visão geral do experimento em fase produtiva.

Apêndice G - Plantas de morangueiro do experimento nos diferentes tratamentos estudados; ASC (adubação solúvel, irrigação controle), ASN1 (adubação solúvel nível de estresse 1), ASN2 (adubação solúvel nível de estresse 2), AAC (adubação alternativa, irrigação controle), AAN1 (adubação alternativa nível de estresse 1), AAN2 (adubação alternativa nível de estresse 2).



Apêndice H - Frutos de morango nos diferentes tratamentos estudados; ASC (adubação solúvel, irrigação controle), ASN1 (adubação solúvel nível de estresse 1), ASN2 (adubação solúvel nível de estresse 2), AAC (adubação alternativa, irrigação controle), AAN1 (adubação alternativa nível de estresse 1), AAN2 (adubação alternativa nível de estresse 2).

Apêndice I - Curva de luz elaborada para determinação da melhor intensidade para posterior realizações das análises das variáveis fotossintéticas utilizando o analisador de gás por infravermelho (IRGA).



Apêndice J - Análise de solo de uma amostragem representativa realizada anteriormente a instalação do experimento.

Análises	Valores
Argila (%)	19
Textura	4
pH H_2O	4.7
Índice SMP	6
Fósforo (mg.L^{-1})	4.5
Potássio (mg.L^{-1})	26
Zinco (mg.L^{-1})	1.53
Cobre (mg.L^{-1})	1.06
Enxofre (mg.L^{-1})	14
Boro (mg.L^{-1})	0.2
Manganês (mg.L^{-1})	36.31
M.O. (%)	1.8
Capacidade de Troca de Cátions Efetivo	4.8
Capacidade de Troca de Cátions pH7	8.5
Saturação bases (%)	48.4
Saturação Alumínio (%)	14.6

Apêndice K - Análise de solo realizada posteriormente o término do experimento.

Análises/ Tratamentos	AA C	AA N1	AA N2	AS C	AS N1	AS N2
Argila (%)	17 ± 1.63	15 ± 1.41	17.5 ± 2.08	15.5 ±1.29	18.75 ± 1.5	16.75 ± 1.5
Textura	4	4	4	4	4	4
pH H_2O	5.5 ± 0.18	5.5 ± 0.17	5.5 ± 0.10	5.22 ± 0.05	5.3 ± 0.05	5.2 ± 0.01
Índice SMP	6.6 ± 0.12	6.2 ± 0.15	6.6 ± 0.10	6.5 ± 0.05	6.4 ± 0.06	6.4 0.08
Fósforo ($mg.L^{-1}$)	69.72 ± 6.04	103 ± 23.77	96.07 ± 15.59	47.87 ± 6.24	40.95 ± 6.89	56.92 ± 7.80
Potássio ($mg.L^{-1}$)	58 ± 5.16	69 ± 8.25	83 ± 14.74	117 ± 22.24	135 ± 19.70	171 ± 14.38
Zinco ($mg.L^{-1}$)	0.92 ± 0.09	1.51 ± 0.60	1.08 ± 0.03	1.45 ± 0.73	0.94 ± 0.13	1.05 ± 0.06
Cobre ($mg.L^{-1}$)	0.80 ± 0.18	1.11 ± 0.27	0.80 ± 0.07	0.76 ± 0.03	0.79 ± 0.07	0.79 ± 0.06
Enxofre ($mg.L^{-1}$)	17.5 ± 4.43	20.75 ± 3.77	17.25 ± 0.9	19.25 ± 1.5	18.25 ± 2.06	17 ± 2.45
Boro ($mg.L^{-1}$)	0.22 ± 0.08	0.15 ± 0.7	0.14 ± 0.09	0.27 ± 0.09	0.22 ± 0.08	0.1 ± 0.01
Manganês ($mg.L^{-1}$)	1.00 ±0.80	3.38 ± 0.58	2.87 ± 0.28	2.64 ± 0.35	2.01 ± 0.43	1.89 ± 0.28
M.O. (%)	1.87 ± 0.05	2.02 ± 0.09	2.12 ± 0.32	1.77 ± 0.05	1.75 ± 0.13	1.9 ± 0.14
Capacidade de Troca de Cátions Efetivo	10.8 ± 0.80	11.15 ± 0.21	11.02 ± 1.34	10.4 ± 0.84	10.9 ± 0.88	11.22 ± 0.52
Capacidade de Troca de Cátions pH7	12.77 ± 0.85	13.52 ± 0.40	13.15 ± 1.24	12.6 ± 0.98	13.38 ± 0.96	13.85 ± 0.53
Saturação bases (%)	83.65 ± 2.98	81.75 ± 2.59	83.3 ± 2.93	80.72 ± 0.73	80.15 ± 0.93	79.85 ± 1.77
Saturação Alumínio (%)	1.25 ± 0.44	0.67 ± 0.16	0.5 ± 0.01	1.25 ± 0.52	1.57 ± 0.39	1.55 ± 0.44