VANUZE COSTA DE OLIVEIRA

BIOFORTIFICATION OF POTATO WITH SELENIUM AND ITS INFLUENCE ON PHYSIOLOGICAL ASPECTS

LAVRAS – MG
2018

VANUZE COSTA DE OLIVEIRA

BIOFORTIFICATION OF POTATO WITH SELENIUM AND ITS INFLUENCE ON PHYSIOLOGICAL ASPECTS

Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Ciência do Solo, área de concentração em Fertilidade do solo e Nutrição de Plantas, para a obtenção do título de Doutora.

Orientador
Dr. Valdemar Faquin

Coorientadora
Drª. Joelma Pereira

LAVRAS – MG
2018

Ficha catalográfica elaborada pelo Sistema de Geração de Ficha Catalográfica da Biblioteca Universitária da UFLA, com dados informados pelo(a) próprio(a) autor(a).

Oliveira, Vanuze Costa de.
Biofortification of potato with selenium and its influence on physiological aspects / Vanuze Costa de Oliveira. - 2018.
93 p. : il.

Orientador(a): Valdemar Faquin.
Coorientador(a): Joelma Pereira.
Tese (doutorado) - Universidade Federal de Lavras, 2018.
Bibliografia.

1. Nutrição mineral de plantas. 2. Biofortificação. 3. Selênio. I. Faquin, Valdemar. II. Pereira, Joelma. III. Título.

VANUZE COSTA DE OLIVEIRA

BIOFORTIFICATION OF POTATO WITH SELENIUM AND ITS INFLUENCE ON PHYSIOLOGICAL ASPECTS

Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Ciência do Solo, área de concentração em Fertilidade do Solo e Nutrição de Plantas, para a obtenção do título de Doutora.

APROVADA em 04 de Maio de 2018.

Dra. Maria Lígia de Souza Silva	UFLA
Dr. Guilherme Lopes	UFLA
Dr. Silvio Júnio Ramos	ITV
Dr. Fabrício William de Ávila	UNICENTRO

Dr. Valdemar Faquin

Orientador

LAVRAS – MG

2018

A Deus, porque d'Ele, por Ele e para Ele são todas as coisas.
Aos meus pais, José Josemar e Margarida. Aos meus irmãos Eliziana, Elizângela, Elivalter, Carlos, Chagas, Jaelson, Verônica, Daniel e Wando.
Aos colegas da turma pioneira em Agroecologia da Universidade Estadual da Paraíba (2008.1) por sua constante luta para o reconhecimento e valorização da ciência Agroecologia.

DEDICO

AGRADECIMENTOS

A Deus por todos os benefícios a mim proporcionados e por sempre estar ao meu lado.

À Universidade Federal de Lavras; Departamento de Ciência do Solo e ao Programa de Pós-graduação em Ciência do Solo.

À Fundação de Apoio à Pesquisa do Estado de Minas Gerais (FAPEMIG), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) pelos recursos destinados à execução dos trabalhos. Ao CNPq pela concessão da bolsa de estudos de doutorado.

Ao professor Valdemar Faquin por todos os ensinamentos repassados, conselhos e orientações, que contribuíram para minha formação profissional e pessoal.

À professora Joelma Pereira por seu auxílio e coorientação. Ao Ramon Batateiro e João Paulo por sua valiosa contribuição.

A todos os técnicos e servidores do Departamento de Ciência do Solo, em especial a Dirce, Lívia, Damiany, Roberto, Geila, João Gualberto e Pezão pelos seus constantes auxílios.

A todos os docentes do Departamento de Ciência do Solo, pelos seus ensinamentos repassados.

Aos professores Maria Lígia de Souza Silva, Guilherme Lopes, Silvio Júnio Ramos e Fabrício William de Ávila pela participação e valiosas contribuições na banca de defesa.

A Karina, Fabrício, Milton, Seu Antônio, Kamila, Paulinha, Olivia, Jéssica, JP, Jefferson, Ediu, por todo auxílio, pela excelente convivência e amizade adquirida.

A todos os colegas e amigos do Departamento de Ciência do Solo e da Universidade Federal de Lavras.

Às colegas de repúblicas: Karla, Karine, Mariane, Isadora, Rafaela, Ester e Emanuely por todos os momentos compartilhados, sejam de alegria, tristeza, perdas ou conquistas.

Aos irmãos em Cristo Jesus, pertencentes à Igreja Cristã Maranata, pelas orações e auxílios nos momentos difíceis.

Aos meus pais, irmãos e familiares por todo o apoio e ensinamentos.

Aos amigos Levi, Kaline, Luciana, Ana Lúcia, Adreani, Thiago Ferreira, Cris, Matheus que apesar da distância sempre se fizeram presente me incentivando e apoiando nos momentos mais difíceis. E que vibraram com minhas conquistas.

RESUMO

O selênio (Se) é um micronutriente para os humanos e animais, compondo diversas selenoproteínas e atuando no sistema antioxidante. Embora sua necessidade de ingestão diária seja baixa, parte da população mundial apresenta deficiência deste micronutriente no plasma sanguíneo. Com o intuito de aumentar a ingestão de Se pela população mundial, diversas pesquisas visando elevar o teor deste elemento em culturas agrícolas têm sido desenvolvidas. Para as plantas o Se não é essencial, porém, baixas doses beneficiam seu sistema antioxidante, enquanto que doses mais elevadas podem ocasionar toxidez, tal como pode ocorrer em humanos e animais. Neste estudo foram realizados dois experimentos com Se em plantas de batata, objetivando avaliar o efeito da aplicação do elemento sobre as trocas gasosas, produção de tubérculos, teor de Se e nutrientes nas plantas e tubérculos, características físico-químicas (teor de sólidos solúveis, acidez titulável, pH e índice de maturação) e atividade antioxidante nos tubérculos. No primeiro experimento, foram aplicadas no solo junto à adubação de plantio duas fontes de Se (selenato e selenito de sódio) e cinco doses de Se (0; 0,75; 1,5; 3,0 e 5,0 mg kg^{-1}), com quatro repetições. No segundo experimento, aplicou-se o Se via foliar durante o período de tuberização das plantas, sendo duas fontes de Se (Selenato e selenito de sódio) e cinco concentrações (0; 25; 50; 75 e 100 μmol L^{-1}), aplicando-se 200 mL por planta, com quatro repetições. No primeiro experimento a aplicação do selenato em baixas doses (0,75 e 1,5 mg kg^{-1}) demonstrou ser a melhor fonte de Se para a biofortificação da batata, pois proporcionou aumento nos teores de Se nos tubérculos sem comprometer o crescimento das plantas, promovendo, também, ganhos na produtividade. Esta fonte também melhorou o sistema antioxidante e reduziu as espécies reativas de oxigênio nos tubérculos. No segundo experimento, o crescimento das plantas bem como a produtividade dos tubérculos não foram afetados pela aplicação do Se via foliar. Entretanto, o Se na forma do selenato elevou a concentração interna de carbono, condutância estomática, assim como proporcionou maiores teores de B na parte aérea das plantas e elevou os teores de K, S, Fe, sólidos solúveis e atividade da SOD e diminuiu o H_2O_2 e a peroxidação lipídica nos tubérculos. O Se na forma do selenito foi superior à do selenato para o teor e acúmulo de Se na parte aérea das plantas e aumentou a concentração de H_2O_2 e malondialdeído (MDA) nos tubérculos. A aplicação foliar de diferentes concentrações de Se, independente da fonte, elevou o seu teor nos tubérculos e ocasionou o aumento da taxa fotossintética nas plantas, índice de maturação e atividade da peroxidase (POD) nos tubérculos. Apesar dos efeitos favoráveis da aplicação do Se nas plantas de batata, ainda são necessários estudos em condições de campo para a comprovação destes resultados, já que fatores ambientais (bióticos e abióticos) podem interferir no crescimento, desenvolvimento e metabolismo das plantas;

buscando-se, assim, estabelecer-se doses ideais para a biofortificação em diferentes regiões do Brasil. Também é fundamental a realização de testes sensoriais para conhecer a aceitação dos alimentos biofortificados por parte dos consumidores.

Palavras-chave: Elemento essencial. Selenato. Selenito. Trocas gasosas. Metabolismo vegetal. Características físico-químicas. Atividade antioxidante.

ABSTRACT

Selenium (Se) is a micronutrient for humans and animals, composing several selenoproteins and acting on the antioxidant system. Although their need for daily intake is low, part of the world population has deficiency of this micronutrient in blood plasma. In order to increase the intake of Se by the world population, several researches aimed at raising the content of this element in agricultural crops have been developed. For plants the Se is not essential, however, low doses benefit your antioxidant system, while higher doses can cause toxicity, as can occur in humans and animals. In this study two experiments were carried out with Se in potato plants, aiming to evaluate the effect of the application of the element on gaseous exchanges, tuber production, Se content and nutrients in plants and tubers, physicochemical characteristics (soluble solids content, titratable acidity, pH and maturation index) and antioxidant activity in the tubers. In the first experiment, two sources of Se (selenate and selenite sodium) and five doses of Se (0; 0.75; 1.5; 3.0 and 5.0 mg kg^{-1}) were applied to the soil together with the planting fertilization, with four replicates. In the second experiment, foliar application was performed during the tuberization period, using two sources of Se (selenate and sodium selenite) and five concentrations (0; 25; 50; 75 and 100 μmol L^{-1}); applying 200 mL per plant, with four replicates. The results of the first experiment showed that the application of selenate in low doses (0.75 e 1.5 mg kg^{-1}) proved to be the best source of Se for the biofortification of the potato, due to having raised the contents of Se in the tubers without compromising the growth of the plants, also promoting gains in productivity. This source and doses also improved the antioxidant system and reduced the reactive oxygen species in the tubers. In the second experiment, the growth of the plants as well as the yield of the tubers were not affected by the foliar application of Se. However, Se in the form of selenate increased the internal carbon concentration, stomatal conductance, as well as increased B contents in the aerial part of the plants and increased the contents of K, S, Fe, soluble solids and SOD activity and decreased the H_2O_2 and lipid peroxidation in the tubers. The Se in the form of selenite was superior to selenate for the content and accumulation of Se in the aerial part of the plants and increased the concentration of H_2O_2 and malondialdehyde (MDA) in the tubers. Foliar application of different concentrations of Se, regardless of the source, increased its content in the tubers and caused an increase in the photosynthetic rate in plants, maturation index and peroxidase activity (POD) in the tubers. Despite the favorable effects of the application of Se in potato plants, field studies are still necessary to prove these results, since environmental factors (biotic and abiotic) may interfere with the growth, development and metabolism of plants; in order to establish ideal doses for biofortification in different regions of Brazil. It is also fundamental to conduct sensory tests to

determine the acceptability of biofortified foods by consumers.

Keywords: Essential element. Selenate. Selenite. Gas exchange. Plant metabolism. Physical-chemical characteristics. Antioxidant activity.

SUMÁRIO

PRIMEIRA PARTE

1 INTRODUÇÃO

O rápido e crescente aumento da população mundial nos últimos anos tem contribuído para a elevação da demanda por alimentos. Atualmente com mais de 7,6 bilhões de pessoas no mundo, a estimativa é que em 2050 este número subirá para 9,7 bilhões de habitantes (ONU, 2017); indicativo de que a demanda por alimentos aumentará significativamente.

Embora a quantidade de alimentos produzidos no Planeta seja suficiente para alimentar a população mundial, a desnutrição tem aumentado atingindo quase metade da população mundial, especialmente mulheres grávidas, adolescentes e crianças (WELCH, 2001; GRAHAM et al., 2007). Isto se deve, em parte, ao melhoramento genético vegetal voltado para ganho em produtividade e desta forma apresentando relação inversa ao conteúdo de minerais em partes comestíveis das plantas (WHITE et al., 2009) e aos baixos teores desses nutrientes nos solos.

Assim, estima-se de quatro a cinco bilhões de pessoas deficientes em ferro (Fe) e dois bilhões anêmicas; 1/5 dessa população deficiente em zinco (Zn); de 0,5 a 1 bilhão deficiente em selênio (Se) e 800 milhões deficientes em iodo (I) (MORAES et al., 2012).

Para o metabolismo humano são necessários 49 nutrientes (RIOS et al., 2009), assim, a baixa ingestão de algum destes pode resultar em sérios problemas na saúde, comprometendo os sistemas imunológico e reprodutor, retardo mental, além de cânceres diversos (ALMONDES et al., 2010; JONES et al., 2017; LOSCALZO, 2014; ZHANG et al., 2017).

Dentre esses nutrientes, encontra-se o selênio (Se) que é um elemento

traço essencial para o metabolismo humano e animal, por ser constituinte de várias selenoproteínas e, no organismo, atuar no sistema antioxidante, imunológico e reprodutor (RAYMAN, 2012). Estudos relatam que a deficiência em Se pode resultar em problemas de saúde como a doença de Keshan (LOSCALZO, 2014), problemas na reprodução (MARCOCCI et al., 2011), oculares (ZHANG et al., 2017) além da estreita relação com diversos tipos de câncer (ALMONDES et al., 2010).

A ingestão de Se varia de acordo com o tipo de alimentação, e com os teores de Se no solo em que os alimentos foram produzidos. Combs (2001) relata que na China, na região de Keshan, o consumo de Se varia entre 7-11 μg por dia; em países europeus, o consumo médio diário é de 30-100 μg, e na América do Norte varia de 60-220 μg por dia. Considerando a necessidade humana de ingestão diária de Se de 50-70 μg por pessoa (UNITED STATES DEPARTMENT OF AGRICULTURE, 2003), infere-se que, em muitas regiões de diversos países, a ingestão de Se está abaixo da recomendada, o que pode acarretar diversos efeitos da deficiência do elemento na saúde humana (MAIHARA et al., 2004).

O teor de um elemento nos alimentos agrícolas e pecuários é dependente da quantidade deste no solo, de sua disponibilidade para as plantas e da espécie vegetal cultivada na área (LYONS et al., 2005). E, no solo, a presença do Se é determinada, principalmente, por fatores geológicos. Solos cujo material de origem são rochas sedimentares, possuem maiores teores de Se, quando comparados àqueles originados de rochas magmáticas (MAYLAND et al., 1989). Fatores edáficos como o pH, potencial redox, textura, ocorrência de óxidos e hidróxidos de ferro e alumínio, clima e vegetação também exercem influência na presença e biodisponibilidade deste elemento no solo (MIKKELSEN; PAGE; BINGHAM, 1989).

Neste sentido, pesquisas relataram que solos do Brasil possuem baixos

teores naturais desse elemento (ABREU et al., 2011; CARVALHO, 2011; GABOS; GOLDBERG; ABREU, 2014), que dentre outros fatores, deve-se à presença de óxidos de Fe e Al e do baixo pH, o que favorece a adsorção do Se, quando na forma do selenito e selenato, assim ocasionando menor disponibilidade do elemento para as plantas. Esse fato explica os baixos teores de Se nos alimentos vegetais cultivados no Brasil (FERREIRA et al., 2002), o que permite inferir a possibilidade de deficiência do elemento na população brasileira, particularmente nos grupos populacionais de baixo poder aquisitivo, que não consomem produtos com altos teores de Se, como peixes e frutos do mar. Tais produtos apresentam o Se na forma orgânica, em que o organismo humano absorve e metaboliza o elemento com maior eficiência (FLORES-MATEO et al., 2006).

A deficiência de Se no organismo humano e animal pode ser contornada através da diversificação da dieta, suplementação alimentar, fortificação por indústria e pelo uso da biofortificação, técnica que consiste no aumento dos teores do elemento nas culturas agrícolas, pela sua introdução na adubação (biofortificação agronômica) ou por melhoramento genético (biofortificação genética) (RIOS et al., 2008; WHITE; BROADLEY, 2009).

A utilização da biofortificação com Se em plantas permite inserir o elemento na cadeia alimentar, tendo as plantas como aliadas (HARTIKAINEN, 2005; LOPES; ÁVILA; GUILHERME, 2017), no sentido de atuarem no controle da ingestão excessiva e, ou, acidental, ocorrente em humanos que fazem uso de suplementos alimentares contendo Se. Também, as formas orgânicas de Se encontradas nos vegetais apresentam maior biodisponibilidade para o organismo humano (VEATCH et al., 2005), resultando no seu maior aproveitamento no metabolismo, ao contrário das formas inorgânicas que são excretadas facilmente pelas fezes e urina.

A estratégia da biofortificação tem sido adotado por alguns países como a

Finlândia (EUROLA et al., 1991) e o Reino Unido (STROUD et al., 2010), países estes que detectaram aumento do teor de Se no plasma sanguíneo da população a partir do enriquecimento das culturas agrícolas com este elemento, outrora deficiente neste micronutriente.

No Brasil, ainda são incipientes pesquisas deste tipo, embora acredite-se que haja uma baixa ingestão de Se pelos brasileiros (FERREIRA et al., 2002). Neste sentido, ao avaliar o nível de Se no plasma sanguíneo em crianças de 3 a 7 anos, estudantes universitários com idade de 20 a 24 anos e idosos com 60 a 95 anos, residentes nos estados de São Paulo, Amapá e Pará, Maihara et al. (2004) constataram que o teor do elemento encontrava-se abaixo do recomendado, exceto para um pequeno grupo de crianças dos estados do Amapá e Pará. Percebendo-se aí a necessidade de se elevar a ingestão de Se por esta população.

Neste sentido, um avanço foi obtido com a publicação da instrução normativa nº 46 (IN 46/2016) pelo Ministério da Agricultura, Pecuária e Abastecimento (MAPA) que no seu artigo 9º sugere que:

> "para os fertilizantes que contenham exclusivamente micronutrientes ou micronutrientes e macronutrientes secundários para aplicação no solo, a garantia mínima do Se nesses produtos não pode ser inferior a 300 mg kg^{-1}" e "as misturas de micronutrientes com fertilizantes mononutrientes, binários e ternários, para as misturas de micronutrientes mais macronutrientes secundários com fertilizantes mononutrientes, binários e ternários para aplicação via solo, via foliar e via fertirrigação e para as misturas exclusivas de micronutrientes com macronutrientes secundários para aplicação via foliar e via fertirrigação deverão possuir no mínimo 30 mg kg^{-1} de Se".

Apesar de não ser essencial para as plantas, diversas pesquisas mostraram que quando o Se é fornecido em baixas doses, ele atua no sistema antioxidante dos vegetais (RAMOS et al., 2010; SCHIAVON et al., 2017; ZHU et al., 2017), aumentando a atividade da superóxido dismutase (SOD), catalase (CAT), ascorbato peroxidase (APX) e peroxidase (POD). O que favorece o aumento da produção de espécies de grande importância no cenário agrícola, como a batata (TURAKAINEN; HARTIKAINEN; SEPPÄNEN, 2004), o arroz (BOLDRIN et al., 2012) e o trigo (BOLDRIN et al., 2016), além da elevação do teor de Se em partes comestíveis destes vegetais.

Embora a SOD, CAT, APX e POD não sejam selenoproteínas, elas são fortemente influenciadas pela presença do Se. E a SOD, em especial, é considerada a primeira linha de defesa contra espécies reativas de oxigênio (EROs), promovendo a dismutação do O_2^{-} em $H_2O_2 + O_2$. O peróxido de hidrogênio (H_2O_2) formado é convertido em $H_2O + O_2$ por uma série de peroxidases por meio da atividade de enzimas como a CAT, APX, POD e PPO, neutralizando, assim, os efeitos deletérios das EROs (NAWAZ et al., 2015).

Além do efeito do Se no sistema antioxidante das plantas, para o sucesso da biofortificação, deve-se considerar a aplicação do elemento em alimentos amplamente consumidos pela população. Neste sentido, a batata (*Solanum tuberosum* L.) quarta cultura agrícola e a olerícola mais produzida e consumida no mundo (TRECHA et al., 2016), possui grande potencial para a inserção do Se na cadeia alimentar dos seres humanos. Além disto, esta cultura apresenta-se como um forte aliado no controle da fome e combate da desnutrição humana, possibilitando o suprimento das necessidades nutricionais diárias. Segundo nutricionistas da FAO, uma dieta composta por batata e leite, pode suprir, em caráter emergencial, todos os nutrientes que o organismo humano necessita para a sua manutenção (ABBA, 2017).

Dentre as cultivares de batata mais comumente plantadas no Brasil

aparece a Ágata que é bastante utilizada devido sua alta aceitação pelos consumidores brasileiros, especialmente para o cozimento. Além disso, os bataticultores optam por esta cultivar devido ser tolerante a diversos patógenos da cultura, resultando em redução nos gastos com insumos para controlar e, ou, combater as pragas e doenças.

Apesar da batata não ser uma planta acumuladora de Se, pesquisas mostraram o sucesso de sua biofortificação, em que, aplicando-se o elemento nas plantas, foi verificada elevação do seu teor nos tubérculos (TURAKAINEN, HARTIKAINEN; SEPPÄNEN, 2004; JEŽEK et al., 2011). Esses resultados indicam que essa cultura biofortificada, devido seu alto consumo e alta aceitação pela população mundial, pode contribuir efetivamente para elevar a ingestão do Se pela população humana no mundo.

O Se pode ser absorvido pelas raízes das plantas em diferentes formas, como selenato (SeO_4^{2-}), selenito (SeO_3^{2-}), compostos orgânicos em aminoácidos, selenometionina e selenocisteína, bem como através das formas voláteis do elemento como di-metil seleneto e di-metil di-seleneto (WHITE et al., 2004), sendo as duas primeiras as mais absorvidas pelos vegetais.

Dentre as principais formas de Se acumuladas nos alimentos estão os aminoácidos seleno-cisteína, seleno-metionina e as formas monometilados *Se*-metil-seleno-cisteína e γ-glutamil-*Se*-metil-seleno-cisteína (KÁPOLNA et al., 2009). Em relação à cultura da batata não foram constatados relatos da forma orgânica predominante.

As formas de Se mais usadas e disponíveis para aplicação nas plantas são o selenato e o selenito. Em programas de biofortificação deve-se levar em conta a fonte de Se aplicada, uma vez que em solos intemperizados, como os das regiões tropicais, com elevadas concentrações de óxidos de Fe e Al na fração argila, há a adsorção específica de ânions, tais como selenato e selenito.

O Se, na forma do selenito, pode ser adsorvido via complexo de esfera

interna, sofrendo adsorção específica com a hematita e goethita (ROVIRA et al., 2008), tal como ocorre com o fosfato em solos tropicais; quanto à adsorção de selenato, sabe-se que ela ocorre em menor intensidade quando comparada ao selenito (LOPES; ÁVILA; GUILHERME; 2017), o que está relacionado ao mecanismo predominante de adsorção que se dá por meio de complexo esfera externa, podendo, também, haver ligações específicas com óxidos e hidróxidos de ferro (PEAK; SPARKS, 2002). Isto resulta em menor disponibilidade de Se para as plantas (ZHANG; SPARKS, 1990).

Ao avaliar a adsorção do Se na forma de selenato em solos do Cerrado Brasileiro, Lessa (2015) observou que aqueles não cultivados adsorveram maior quantidade de Se, em relação aos cultivados. O pesquisador relacionou esta menor adsorção de Se nos solos cultivados ao manejo e aplicação de fertilizantes à base de enxofre (na forma do sulfato) e fósforo (como fosfato), que apresentam características químicas semelhantes ao selenato e selenito, respectivamente, resultando na ocupação dos sítios de adsorção pelos ânions aplicados na adubação (sulfato e fosfato) e promovendo menor adsorção do selenato.

Assim como no solo, no interior das plantas, as formas de Se também diferem quanto à absorção e mobilidade, sendo o selenato mais facilmente transportado para a parte aérea pelos vasos do xilema, enquanto que o selenito tende a se acumular nas raízes (ZHANG et al., 2003). O menor transporte de selenito para a parte aérea está relacionado à sua conversão acelerada para formas orgânicas de Se, como selenometionina (ZAYED; LYTLE; TERRY, 1998), as quais são armazenadas nas raízes.

Diversos trabalhos mostraram que a aplicação de selenato foi mais eficiente que a aplicação de selenito para aumentar a concentração de Se em plantas (BOLDRIN et al., 2012; BOLDRIN et al., 2013; CARTES; GIANFREDA; MORA, 2005; RAMOS et al., 2011). Excluindo-se as condições de solo e suas características, trabalhos conduzidos em solução nutritiva também

demonstram maior acúmulo de Se ao se aplicar o selenato em plantas quando comparado à aplicação de selenito (RAMOS et al., 2011).

Para a cultura da batata não há estudos neste sentido (comparação entre estas duas fontes de Se: selenato e selenito para a biofortificação desta espécie vegetal). Considerando que o selenito tende a acumular-se nas raízes das plantas e que os tubérculos da batata são a parte comestível da espécie, percebe-se a necessidade de estudos a respeito da aplicação do Se na batata, buscando-se, desta maneira, identificar a melhor fonte para elevar o teor do elemento nos tubérculos.

Assim como as fontes de Se, as formas de aplicação do elemento nas plantas (via solo ou via foliar), também afetam seu acúmulo nas partes comestíveis dos vegetais. Hu et al. (2002), em um experimento realizado para avaliar métodos de aplicação de Se para a biofortificação do arroz, utilizando um fertilizante enriquecido com Se, aplicado no solo e via foliar, verificaram as diferentes formas de aplicação de Se não afetou a produção de grãos da cultura. Entretanto, verificou-se que os métodos de aplicação do Se proporcionaram elevação do seu teor nos grãos.

Também em arroz, Boldrin et al. (2013) estudando fontes (selenato e selenito) e locais de aplicação (solo e foliar) de Se, observaram aumento na produção de grãos com a aplicação foliar de ambas as fontes e aumento nos teores de Se nos grãos quando utilizado o selenato, sendo a aplicação via solo mais efetiva.

Embora pesquisas envolvendo a biofortificação de várias espécies vegetais com o Se tenham sido desenvolvidas nas últimas décadas no mundo, no Brasil e em países de clima tropical, estes estudos ainda são bastante recentes. Diante disto, trabalhos com a aplicação do Se em plantas em condições de clima tropical, como o Brasil são relevantes, já que esta prática pode favorecer a implantação de um programa de biofortificação de alimentos vegetais não só no

País, mas também em outros que apresentem características edafoclimáticas semelhantes.

Diante disto, este trabalho objetivou avaliar o efeito da aplicação (via solo e foliar) de fontes inorgânicas de Se (selenato e selenito) e doses de Se em plantas de batata cultivadas em solo tropical (Latossolo Vermelho-Amarelo distrófico de textura média) e ambiente protegido. Buscando-se confirmar as hipóteses de que a aplicação de baixas doses de Se aumenta o teor desse elemento nos tubérculos e melhoram características produtivas, fisiológicas e bioquímicas da batata; e que a fonte inorgânica e o local de aplicação do Se exercem influência para o sucesso da biofortificação da batata.

A avaliação do efeito do Se em características fisiológicas e físico-químicas da batata justifica-se devido este elemento apresentar efeito hormese em plantas (sendo benéfico em baixos doses e tóxico quando altas doses ou concentrações são fornecidas), assim como ocorre em humanos. Ademais, este elemento pode atuar no sistema antioxidante das plantas que está diretamente ligado à maturação e senescência do vegetal (SINGH; DWIVEDI, 2008), especialmente quando em altas doses, ocasionando efeito pro-oxidante (HARTIKAINEN et al., 2000), o que resulta em danos nas membranas celulares e consequente interferência nas trocas gasosas das plantas, por meio de processos adaptativos dos vegetais, como elevação do número e densidade de estômatos (FRANKS; DRAKE; BEERLING, 2009).

Neste contexto, a tese está dividida em dois artigos já submetidos em periódicos científicos. O primeiro, intitulado "Physiological and physicochemical responses of potato to selenium biofortification in tropical soil" refere-se à aplicação de fontes inorgânicas (selenato e selenito de sódio) e doses de Se (0; 0,75; 1,5; 3,0 e 5,0 mg kg^{-1}) no solo, juntamente com a adubação de plantio. O segundo artigo com título "Agronomic biofortification of potato with selenium applied via foliar in tropical conditions" é referente à aplicação foliar

de fontes inorgânicas (selenato e selenito de sódio) e concentrações de Se (0; 25; 50; 75 e 100 μmol L^{-1}) em plantas de batata na fase de tuberização da cultura.

REFERÊNCIAS

ABBA, Associação Brasileira da Batata, 2017. **A batata/História da batata**. Disponível em: http://www.abbabatatabrasileira.com.br/2008/abatata.asp?id_BAT=2. Acesso em janeiro de 2017.

ABREU, L. B. et al. Sorção de selênio em solos do bioma cerrado. **Revista Brasileira de Ciência do solo**, Viçosa, v. 35, n. 6, p. 1995–2003, nov./dez. 2011.

ALMONDES, K. G. S. et al. O papel das selenoproteínas no câncer. **Revista da Associação Medica Brasileira**, São Paulo, v. 56, n. 4, p. 484–488, jul./aug. 2010.

BOLDRIN, P. F. et al. Selenato e selenito na produção e biofortificação agronômica com selênio em arroz. **Pesquisa Agropecuária Brasileira**, Brasília, v. 47, n. 6, p. 831-837, jun. 2012.

BOLDRIN, P. F. et al. Soil and foliar application of selenium in rice biofortification. **Journal of Food Composition and Analysis**, San Diego, v. 31, n. 2, p. 238–244, sep. 2013.

BOLDRIN, P. F. et al. Selenium promoters sulfur accumulation and plant growth in wheat (*Triticum aestivum*). **Physiologia Plantarum**, Denmark, v. 158, n. 1, p. 80–91, sep. 2016.

CARTES, P.; GIANFREDA, L.; MORA, M. L. Uptake of selenium and its antioxidant activity in ryegrass when applied as selenate and selenite forms. **Plant and Soil**, The Hague, v. 276, n. 1-2, p. 359-367, oct. 2005.

CARVALHO, G. S. **Selênio e mercúrio em solos sob cerrado**. 2011. 93 p. Tese (Doutorado em Ciência do Solo) – Universidade Federal de Lavras, Lavras, 2011.

COMBS, G. F. Selenium in global food systems. **British Journal of Nutrition**, Crambridge, v. 85, n. 5, p. 517-547, may 2001.

EUROLA, M. et al. Results of the finnish selenium monitoring program 2000-2001. In: MTT AGRIFOOD RESEARCH FINLAND. **Agrifood Research Reports 36**. Jokioinen, 2003. 42p.

FERREIRA, K. S. et al. Concentrações de selênio em alimentos consumidos no

Brasil. **Revista Panamericana de Salud Pública**, Washington, v. 11, n. 3, p. 172–177, 2002.

FLORES-MATEO, G. et al. Selenium and coronary heart disease: a meta-analysis. **American Journal of Clinical Nutrition**, Boston, v. 84, n. 4, p. 762-773, oct. 2006.

FRANKS, P. J.; DRAKE, P. L.; BEERLING, D. J. Plasticity in maximum stomatal conductance constrained by negative correlation between stomatal size and density: An analysis using *Eucalyptus globulus*. **Plant, Cell & Environment,** v. 32, n. 12, p. 1737-1748, nov. 2009.

GABOS, M. B.; ALLEONI, L. R. F.; ABREU, C. A. Background levels of selenium in some selected Brazilian tropical soils. **Journal of Geochemical Exploration**, Amsterdam, v. 145, p. 35-39, oct. 2014.

GRAHAM, R. D. et al. Nutritious subsistence food systems. **Advances in Agronomy**, New York, v. 92, n. 4, p.1-74, may. 2007.

HARTIKAINEN, H. Biogeochemistry of selenium and its impact on food chain quality and human health. **Journal of Trace Elements in Medicine and Biology**, Stuttgart, v. 18, n. 4, p. 309-318, jun. 2005.

HU, Q. et al. Determination of selenium concentration in rice and the effect of foliar application of Se-enriched fertiliser or sodium selenite on the selenium content of rice. **Journal of the Science of Food and Agriculture**, London, v. 82, n. 8, p. 869–872, may. 2002.

JEŽEK, P. et al. Effect of foliar application of selenium on the content of selected amino acids in potato tubers (*Solanum tuberosum* L.). **Plant, Soil and Environment**, Praha, v. 57, n. 7, p. 315–320, jan.2011.

JONES, G. D. et al. Selenium deficiency risk predicted to increase under future climate change. **Proceedings of the National Academy of Sciences**, Washington, v. 114, n. 11, p. 2848–2853, jan. 2017.

KÁPOLNA, E. et al. Effect of foliar application of selenium on its uptake and speciation in carrot. **Food Chemistry**, London, v. 115, n. 4, p. 1357-1363, aug. 2009.

LESSA, J. H. L. **Adsorção e dessorção de selênio em solos cultivados e não cultivados sob Cerrado**. 2015. 66 p. Dissertação (Mestrado em Ciência do Solo) – Universidade Federal de Lavras, Lavras, 2015.

LOSCALZO, J. Keshan disease, selenium deficiency, and the selenoproteome. **New England Journal of Medicine**, Waltham, v. 370, n. 18, p. 1756-1760, may. 2014.

LOPES, G.; ÁVILA, F. W.; GUILHERME, L. R. G. Review - Selenium behavior in the soil environment and its implication for human health. **Ciência e Agrotecnologia**, Lavras, v. 41, n. 6, p. 605-615, nov./dec. 2017.

LYONS, G. et al. Selenium concentration in wheat grain: is there sufficient genotypic variation to use in breeding? **Plant and Soil**, The Hague, v. 269, n. 1/2, p. 369-380, feb. 2005.

MAIHARA, V. A. et al. Daily dietary selenium intake of selected Brazilian population groups. **Journal of Radioanalytical and Nuclear Chemistry**, Budapest, v. 259, n. 3, p. 465-468, jul./sep. 2004.

MARCOCCI, C. et al. Selenium and the Course of Mild Graves' Orbitopathy. **New England Journal of Medicine**, Waltham, v. 364, n. 20, p. 1920-1931, may. 2011.

MAYLAND, H. F. et al. ln: L.W. Jacobs (ed). Selenium in Agriculture and the Environment. **Soil Science Society of America**, Madison, Special Publication. n. 23, apr. 1989.

MIKKELSEN, R.; PAGE, A. L.; BINGHAM, F. T. Factors affecting selenium accumulation by agricultural crops. In: Selenium in agriculture and the Environment. **Soil Science Society of America**, Madison, Special Publication, v. 23, p. 65–94, 1989.

MORAES, M. F. et al. Biofortificação – alternativa à segurança nutricional. **Informações Agronômicas**, IPNI, Piracicaba, n. 140, dez. 2012.

NAWAZ, F. et al. Effect of selenium foliar spray on physiological and biochemical processes and chemical constituents of wheat under drought stress. **Ecotoxicology Environmental Safety**, Amsterdam, v. 113, p. 191-200, mar. 2015.

ONU - Organização das Nacões Unidas. **World Population Prospects: key**

findings and advance tables (2017 revision). New York: United Nations, 2017.

PEAK, D.; SPARKS, D. L. Mechanisms of selenate adsorption on iron oxides and hydroxides. **Environmental Science and Technology**, Washington, v. 36, n. 7, p. 1460-1466, jan./feb. 2002.

RAMOS, S. J. et al. Selenium biofortification and antioxidante activity in lettuce plants fed with selenate and selenite. **Plant, Soil and Environment**, Praha, v. 56, p. 584-588, may. 2010.

RAMOS, S. J. et al. Selenium accumulation in lettuce germplasm. **Planta**, Berlin, v. 233, n. 4, p. 649–660, apr. 2011.

RAYMAN, M. P. Selenium and human health. **Lancet**, v. 379, p. 1256–68, feb. 2012.

RIOS, S. A. et al. Biofortificação: culturas enriquecidas com micronutrientes pelo melhoramento genético. **Revista Ceres**, Viçosa, v. 56, n. 6, p. 713–718, nov./dec. 2009.

ROVIRA, M. et al. Sorption of selenium (IV) and selenium (VI) onto natural iron oxides: goethite and hematite. **Journal of Hazardous Materials**, Amsterdam, v. 150, n. 2, p. 279-284, jan. 2008.

SCHIAVON, M. et al. Selenium fertilization alters the chemical composition and antioxidant constituents of tomato (*Solanum lycopersicon* L.). **Journal of Agricultural and Food Chemistry**, Washington, v. 61, p. 542–554, nov. 2013.

SINGH, R.; DWIVEDI, U. N. Effect of ethrel and 1-methylcyclopropene (1-MCP) on antioxidants in mango (*Mangifera indica* var. Dashehari) during fruit ripening. **Food Chemistry**, England, v. 111, n. 4, p. 951-956, dec. 2008.

STROUD, J. L. et al. Soil factors affecting selenium concentration in wheat grain and the fate and speciation of Se fertilizers applied to soil. **Plant and Soil**, The Hague, v. 332, p. 31-40, dec. 2010.

TRECHA, C. O. et al. Influência da safra de verão e outono no dano de larvas da vaquinha em tubérculos de batata cultivadas em casa de vegetação. **Revista de la Facultad de Agronomía**, La Plata, v. 115, n. 1, p. 1-8, jan. 2016.

TURAKAINEN, M.; HARTIKAINEN, H.; SEPPÄNEN, M. M. Effects of selenium treatments on potato (*Solanum tuberosum* L.) growth and concentrations of soluble sugars and starch. **Journal Agricultural and Food**

Chemistry, Washington, v. 52, n.1, p.5378–5382, aug. 2004.

UNITED STATES DEPARTMENT OF AGRICULTURE. **Dietary reference intakes:** elements. Washington: [s.n.], 2003. Disponível em: <http://www.iom.edu/Global/News--%20Announcements/~/media/48FAAA2FD9E74D95BBDA2236E7387B49.ash>. Acesso em: 15 dez. 2017.

VEATCH, A. E. et al. Selenium and nutrition: The accuracy and variability of the selenium content in commercial supplements. **Journal of Radioanalytical and Nuclear Chemistry**, Budapest, v. 264, n. 1, p. 33-38, mar. 2005.

WELCH, R. M. **Micronutrients, agriculture e nutrition: linkages for improved health and well being**. In; Singh, K.; Mori, S.; Welch, R.M. (Ed.). Perspectives on the micronutrients nutrition of crops. Jodhpur: Scientific Publichers, 2001. p. 247-289.

WHITE, P. J. et al. Interactions between selenium and sulphur nutrition in *Arabidopsis thaliana.* **Journal of Experimental Botany**, Oxford, v. 55, n. 404, p. 1927–1937, aug. 2004.

WHITE, P. J.; BROADLEY, M.R. Biofortification of crops with seven mineral elements often lacking in human diets - iron, zinc, copper, calcium, magnesium, selenium and iodine. **New Phytologist**, Lancaster, v. 182, n. 1, p. 49-84, jan. 2009.

ZAYED, A.; LYTLE, C. M.; TERRY, N. Accumulation and volatilization of different chemical species of selenium by plants. **Planta**, Berlin, v. 206, n. 2, p. 284-292, oct. 1998.

ZHANG, P. C.; SPARKS, D. L. Kinetics of selenate and selenite adsorption/desorption at the goethite/water interface. **Environmental Science & Technology**, Easton, v. 24, n. 12, p. 1848-1856, dec. 1990.

ZHANG, Y. et al. Uptake and transport of selenite and selenate by soybean seedlings of two genotypes. **Plant and Soil**, The Hague, v. 253, n. 2, p. 437-443, jun. 2003.

ZHANG, G. et al. Genetic associations with gestational duration and spontaneous preterm birth. **New England Journal of Medicine**, Waltham, v. 377, n. 12, p. 1156-1167, sep. 2017.

ZHU, Z. et al. Selenium delays tomato fruit ripening by inhibiting ethylene biosynthesis and enhancing the antioxidant defense system. **Food Chemistry**, England, v. 219, n. 1, p. 179-184, mar. 2017.

SEGUNDA PARTE

ARTIGO 1

Physiological and physicochemical responses of potato to selenium biofortification in tropical soil

(Artigo submetido ao periódico *Potato Research*)

Physiological and physicochemical responses of potato to selenium biofortification in tropical soil

Vanuze Costa de Oliveira[1,*] · Valdemar Faquin[1] · Fabrício Ribeiro Andrade[2] · João Paulo Carneiro[1] · Ediu Carlos da Silva Júnior[1] · Kamila Rezende Dázio de Souza[3] · Joelma Pereira[4] · Luiz Roberto Guimarães Guilherme[1]

Abstract The technique of biofortification with Se in several agricultural crops has been well researched by the scientific community, however, for vegetable crops cultivated in tropical conditions there is still little information. The objective of this study was to evaluate the effect of the application of Se in the soil on physiological characteristics, agronomic, biofortification and antioxidant enzyme activity in *Solanum tuberosum* L. grown in tropical soil. Potato plants (cv. Agata) were cultivated in pot (7 kg) with soil that received two sources of Se (selenate and selenite) and five doses of Se (0; 0.75; 1.5; 3.0 e 5.0 mg kg^{-1}). The results showed that the application of Se in the soil by both sources increases its content in the tubers. When applied in small doses, the Se provides beneficial effects on the production of tubers, increases Ca content in shoots and activates enzymes of the antioxidant system. High doses of Se, in addition to decreasing production, reduce S content, pH and peroxidase activity in the tubers. Thus, selenate is the most efficient source for biofortification of potato under tropical conditions when

(✉) Vanuze Costa de Oliveira
vanuze.costa@gmail.com, Orcid: 0000-0001-8979-5484
[1] Departament of Soil Science, Federal University of Lavras, Lavras - MG, Brazil
[2] Federal Institute of Mato Grosso, Juína - MT, Brazil
[3] Department of Biology, Federal University of Lavras, Lavras - MG, Brazil
[4] Departament of Food Science, Federal University of Lavras, Lavras - MG, Brazil

supplied in low doses.

Keywords: selenate, selenite, post-harvest quality, antioxidant activity.

Introduction

Selenium (Se) is an essential element for animal and human metabolism by make part of selenoproteins, being a component of glutathione peroxidase and acting on the organism antioxidant system (Rayman 2012). Se deficiency affects hundreds of millions of people around the world (White and Brown 2010). However, this lack of Se can be overcome through a diversified diet, food supplementation, fortification by industry and the consumption of biofortified food obtained through the technique consisting in increasing the element content in edible parts of the plant, named biofortification.

This technique is ideal to insert the element in the food chain because the plants act in an effective way to control the excessive intake, which can occur in humans through the use of dietary supplements. In addition, Se ingested in organic form is more easily metabolized and utilized by the body (Li et al. 2008).

For plants, the Se is not essential, however, when applied at low doses, it is beneficial for some groups of plants by increasing the activity of the enzymatic system (Zhu et al. 2004). In addition, the antioxidant properties of these enzymes are similar to that of other antioxidants, such as superoxide dismutase, catalase and ascorbate peroxidase, and thereby decreases reactive oxygen species and lipid peroxidation.

Considering that most plants, especially those of agricultural interest, are sensitive to high doses of Se (Boldrin et al., 2012) and the range between the beneficial and phytotoxic doses is very narrow (Pilon-Smits et al., 2009), care should be taken when

setting doses to apply this element.

The inorganic source of Se to be used for biofortification is also a key factor for the success of biofortification, because selenate and selenite are absorbed and metabolized differently in plants (Zhang et al. 2003; Ramos et al. 2010). Researchers have shown that either via soil or via nutritive solution, selenate presents higher efficiency for biofortification of potato (Turakainen et al. 2004), lettuce (Ramos et al. 2010) and rice (Boldrin et al. 2012), when compared to selenite.

The success of biofortification also depends on the target crop, selecting a crop widely consumed by the population, such as potato (*Solanum tuberosum* L.), the fourth agricultural species more cultivated and consumed in the world (FAO 2013). In addition, it is consist of several essential elements for human health such as potassium, carbohydrates and vitamins. What makes potatoes a potential agricultural product to be biofortified. In addition, studies showed that the application of Se via soil and leaves improved physiological and agronomic characteristics of the potato crop and increased Se content in the tubers (Turakainen et al. 2004; Ježek et al. 2011).

In this context and considering that there are no reports on the biofortification of potato with Se cultivated in tropical soil conditions like those of Brazil, the aim of this study was to evaluate the effect of different sources and doses of Se in physiological characteristics, agronomic, of biofortification, nutrition and enzymatic activity of the antioxidant system in this crop.

Material and methods

Plant material and experimental design

The experiment with potato (*Solanum tuberosum*), cultivar Ágata, was conducted from December 2016 to April 2017 under protected environmental conditions at the Department of Soil Science of the Federal University of Lavras, Lavras-MG, Southeastern Brazil (21°22'62"S, 44°97'94"W and altitude of 918 m). The cultivar Ágate was used, because it is one of the most cultivated in Brazil, due to its wide possibility of consumption in the form of purees, fried or cooked.

Throughout the crop cycle, minimum and maximum temperatures were, on average, 20 and 33 °C, respectively, and relative humidity of 67%. Pots with 7 kg filled with dystrophic Red-Yellow Latosol (LVAd) collected in the layer of 0-0.20 m were used.

The soil was chemically and physically characterized according to Embrapa (2011): pH-H_2O = 4.8; P (Mehlich-1) = 1.1 mg dm^{-3}; P-rem = 26.6 mg L^{-1}; K = 32 mg dm^{-3}; Ca = 0.3 $cmol_c$ dm^{-3}; Mg = 0.1 $cmol_c$ dm^{-3}; Al = 0.6 $cmol_c$ dm^{-3}; H+Al = 4.5 $cmol_c$ dm^{-3}; SB = 0.5 $cmol_c$ dm^{-3}; OM = 16 g kg^{-1}; V = 9.6%; t = 1.1 $cmol_c$ dm^{-3}; T = 5.0 $cmol_c$ dm^{-3}; m = 55.5%; S = 9.9 mg dm^{-3}; Zn = 0.5 mg dm^{-3}; B = 0.2 mg dm^{-3}; Fe = 41.6 mg dm^{-3}; Mn = 4.1 mg dm^{-3}; Cu = 0.5 mg dm^{-3}; clay = 710 g kg^{-1}; silt = 140 g kg^{-1} and sand = 150 g kg^{-1}. The natural concentration of Se in the soil was 0.065 mg dm^{-3}.

Based on the soil analysis, the basis saturation was increased to 60% using dolomite limestone (CaO = 37%, MgO = 15% and CCE = 85%). The soil remained incubated for 30 days with humidity close to 60% of the total pore volume (TPV). Fertilization was performed according to Malavolta (1980) modified, being 380 mg of N and 400 mg of K (divided into four applications); 350 mg P; 50 mg S; 0.5 mg B; 1.5 mg Cu; 5 mg Zn and 0.1 mg Mo per kg of soil.

The experimental design was completely randomized, in a 5 x 2 factorial scheme,

with five doses of Se (0; 0.75; 1.5; 3.0 e 5.0 mg kg^{-1}, applied together with the planting fertilization) and two sources of Se (sodium selenate-Na_2SeO_4 and sodium selenite-$Na_2SeO_35H_2O$, Sigma-Aldrich, Saint Louis, USA), with four replicates, totaling 40 plots. Each experimental unit consisted of one pot containing one potato plant. In all experimental period, soil moisture was maintained close to the field capacity, with application of deionized water.

Gas exchange

At 80 days after sowing (DAS) the gas exchanges were evaluated by measuring the net assimilation rate of CO_2 (*A*-µmol CO_2 m^{-2} s^{-1}), stomatal conductance (*gs*-mol H_2O m^{-2} s^{-1}), transpiration (*E*-mmol H_2O m^{-2} s^{-1}) and internal CO_2 concentration (*Ci*-mmol CO_2 m^{-2} s^{-1}), using a portable infra-red photosynthesis analyzer (Infra Red Gas Analyzer - IRGA, model Li-6400XT, LI-COR, Nebraska, USA).

The readings were performed on a day without cloud, between 9:30 and 12:00, with an average temperature of 28 °C and a relative humidity of 60%.

Shoot dry mass and fresh tubers production

At 105 DAS the irrigation was suspended for the desiccation of the plants and after seven days, when verified the complete drying of the branches, the harvesting and separation of the shoot and tubers were done. The shoot was dried in an air circulation oven at 70 °C until constant weight, obtaining the dry mass of the shoots, and the tubers were washed and weighed to obtain the yield.

Determination of Se and nutrients in shoots and tubers

For the analyses of Se and nutrients contents, five tubers were cut and dried in an air circulation oven at 70 °C until constant weight. Then the plant tissues were milled in a Willey type mill with mesh sieves of 1.0 mm (40 mesh).

For the determination of Se content in the shoots and tubers, the USEPA method 3051A (USEPA 1998) was used. 0.5 g of plant material (in duplicate) were digested in 5 mL of nitric acid in a microwave oven (Mars 5CEM Corporation, Matthews, USA). For the quality control of analysis, in each digestion batch two samples were placed with standard reference material (White Clover - BCR 402, Institute for Reference Materials and Measurements, Geel, Belgium) with known content of Se (6.7 mg kg^{-1}). The mean recovery for Se in the SRM was 90%. The extracts were analysed by atomic absorption spectrometry with electrothermal atomization in graphite furnace (Perkin Elmer, model AA-analyst 800, Midland, Canada).

The digestion and determination of macro and micronutrients contents in shoots and tubers were carried out according to methodology described by Malavolta et al. (1997). The contents of Se, macronutrients and micronutrients were expressed in dry mass of plant tissues. The accumulation of Se was determined by multiplying the weight of the dry mass (divided by 1000) with the content of the element obtained in the respective plant tissues.

Physicochemical and biochemical analyses in tubers

After harvesting, five tubers of each plot were stored in liquid nitrogen and kept at -80 °C for subsequent physicochemical and biochemical analysis. The antioxidant activity of

superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), polyphenol oxidase (PPO), pH, soluble solids content (SS), titratable acidity (AT), maturation index, concentration of hydrogen peroxide (H_2O_2) and MDA (lipid peroxidation) were determined.

The pH was determined according to the methodology proposed by the AOAC (2010), using a digital potentiometer (Digimed, model DM-20). The quantification of soluble solids occurred according to the methodology proposed by Braun et al. (2010), with analysis performed in an electronic refractometer (Atago, model PR100). The titratable acidity determination was carried out according to AOAC (2010). By the ratio between SS and TA, the maturation index was obtained (AOAC 2010).

The extract for analysis of SOD, CAT and APX activity was obtained according to Biemelt et al. (1998). For this, 0.2 g of the tubers were macerated in liquid nitrogen and 22 mg of polyvinylpolypyrrolidone (PVPP), then 1.5 mL of the buffer was added, consisting of 375 µL of potassium phosphate 100 mmol L^{-1} (pH 7.8), 15 µL of 0.1 mmol L^{-1} EDTA, 75 µL of 10 mmol L^{-1} ascorbic acid and 1035 µL of water. The extract was centrifuged at 13000 g for 10 minutes at 4 °C, then the supernatant was collected and stored at -80 °C.

For the determination of SOD activity, 5 µL of the enzyme extract plus 5 µL of water were added to 171 µL of the mix composed by potassium phosphate 50 mmol L^{-1} (pH 7.8), methionine (14 mmol L^{-1}), EDTA (0.1 µmol L^{-1}), NBT (75 µmol L^{-1}) and riboflavin (2 µmol L^{-1}). Then the reaction medium together with the sample remained illuminated by a 15W fluorescent lamp for 7 minutes, according to Giannopolitis and Ries (1977), with modifications. The readings were performed using absorbance of 560 nm, and

measured using a spectrophotometer (Epoch-Bioteck-Elisa).

Catalase (CAT) activity was determined by adding aliquots of 4.5 µL of the enzyme extract, plus 4.5 µL of water to 171 µL of the incubation buffer, containing 90 µL of 100 mmol L^{-1} potassium phosphate (pH 7.0), 9 µL of hydrogen peroxide (250 mmol L^{-1}) and 72 µL of distilled water and incubated at 30 °C, according to the methodology proposed by Havir and McHale (1987) with modifications. The reading was performed at 240 nm, using spectrophotometer (Epoch-Bioteck-Elisa).

For the measurement of APX activity, 4.5 µL aliquots of the extract plus 4.5 µL of water were added to 171 µL of incubation buffer containing 90 µL of potassium phosphate 200 mmol L^{-1} (pH 7.0), 9 µL of ascorbic acid (10 mmol L^{-1}), 9 µl of H_2O_2 (2 mmol L^{-1}) and 63 µl of distilled water. With the reading performed in absorbance at 290 nm (Nakano and Asada 1981), using a spectrophotometer (Epoch-Bioteck-Elisa).

For the determination of the POD and PPO activity, the enzymes were extracted according to Matsumo and Uritani (1972). The extracts were read at 470 and 395 nm absorbance for POD (Matsumo and Uritani, 1979) and PPO (Teisso, 1979), respectively. For this, a spectrophotometer was used (Femto, model 600 Plµs).

For the quantification of hydrogen peroxide, 0.2 g of the tubers were macerated in liquid nitrogen and PVPP, being homogenized in 1.5 mL of 0.1% trichloroacetic acid (TCA). Then the mixture was centrifuged at 12,000 g for 15 minutes at 4 °C. The H_2O_2 was quantified by measuring the absorbance at 390 nm in a reaction composed of 45 µL of the extract, 45 µL of potassium phosphate (10 mmol L^{-1}) and 90 µL of potassium iodide (1 mol L^{-1}), according to Velikova et al. (2000). The reading was performed in a spectrophotometer (Epoch-Bioteck-Elisa).

The determination of lipid peroxidation (MDA content) was performed according to methodology proposed by Buege and Aust (1978). Aliquots (125 μL) were added to the reaction medium composed of thiobarbituric acid (0.5%) and trichloroacetic acid (10%), following incubation at 95 °C for 30 minutes, the reaction was stopped by ice cooling. The readings were performed in a spectrophotometer at 535 and 600 nm using a spectrophotometer (Epoch-Bioteck-Elisa).

Statistical analyzes

All data were submitted to analysis of variance ($p \leq 0.05$) and, when verified statistical difference by F test, the Scott-Knott test (Scott and Knott, 1974) and polynomial regression analysis were applied using the statistical program R 3.2.3 (RDCT 2015). The graphics were produced using SigmaPlot 12.5 software (Systat Software, Inc., San Jose, CA - USA).

Results and discussion

Gas exchange

Stomatal conductance (*gs*) was influenced by interaction between sources and doses of Se whereas leaf transpiration (*E*) was altered by Se doses (Fig. 1). The photosynthetic rate (*A*) and the internal carbon concentration (*Ci*) were not altered by the treatments (data not presented).

The application of Se as selenite increased the *gs* in 106% and 176% in the doses of 3.0 and 5.0 mg kg^{-1}, respectively. The lowest doses did not differ statistically from the control, as well as the application of the selenate at all doses (Fig. 1a). The *E* reduced

with the lowest doses of Se, observing an increase in the highest dose applied (5.0 mg kg^{-1}), which did not differ from the control (Fig. 1b). Alyemeni et al. (2017) detected increase at *gs* and *E* in tomato plants grown in nutrient solution with low concentration of Se (10 μM) in the form of selenite and Nawaz et al. (2015) detected an increase in gas exchange in wheat by foliar application of 40 g ha^{-1} of Se as selenate. According to the researchers, this increase in the gas exchange of these species is due to the performance of Se in the protection of photosynthetic devices, when this element is supplied in low concentrations.

In the present research with potato, the increase in the *gs* and *E* of the plants, when applied 5.0 mg kg^{-1} of Se, is due to possible damages in the guards cells caused by the increase of reactive oxygen species (ROS) and lipid peroxidation, resulting from the pro-oxidant effect of Se when supplied to plants at high doses (Hartikainen et al. 2000). As an adaptation to stress, changes in leaf anatomy can have occurred, as an increase in stomatal density, resulting in higher gs and thus allowing elevation of the *E* due to the higher number of stomata.

Production of shoot dry mass and fresh tubers

Shoot dry mass (SDM) production and fresh tubers were affected by Se doses, regardless of the source applied (Fig. 2). SDM showed maximum production (19.33 g pot^{-1}) at the estimated dose of 3.66 mg kg^{-1} of Se (Fig. 2a), with an increase of approximately 22% in relation to the control.

Researches have shown that in low doses, Se increased dry matter production in potato, lettuce, rice, garlic, and wheat plants (Turakainen et al. 2004; Boldrin et al. 2012;

Boldrin et al. 2016). This fact may be related to the increase of the antioxidant activity in the cells and to the consequent reduction of reactive oxygen species (Schiavon et al. 2017).

As for the tubers, the highest yield was obtained at the dose of 0.75 mg kg^{-1} of Se, giving an increase of 4% in relation to the control, independent of the Se source (Fig. 2b). However, at the highest dose (5.0 mg kg^{-1} of Se) there was a 17% reduction in tuber production. This fact may be related to the pro-oxidant action of Se when applied in high doses, causing an increase in H_2O_2 concentration and lipid peroxidation (Ramos et al. 2010 and 2012; Puccinelli et al. 2017a).

Content and accumulation of Se

Interaction between sources and doses of Se was observed for the content and accumulation of Se in the shoots and tubers (Fig. 3). The highest Se content in shoot was obtained with the application of selenite (6.20 mg kg^{-1} of Se) and selenate (5.63 mg kg^{-1} of Se) for the doses estimated at 3.95 and 3.70 mg kg^{-1} of Se, respectively (Fig. 3a).

In the tubers, the Se content increased linearly with the increase of the doses applied for both sources, being an increase of 5000% and 1400%, for selenate and selenite respectively, in the dose of 5.0 mg kg^{-1} of Se in relation to the control (Fig. 3b). These results corroborate those from previous researches, in which the application of Se increased its content in the edible part of agricultural species (Boldrin et al. 2016; Puccinelli et al. 2017b; Zhu et al. 2017), especially when applied in the form of selenate.

For the purposes of biofortification, the Se content in the edible parts of the vegetable should be considered, in the case of potato, the tubers. The minimum daily requirement

for Se ingestion is 70 μg for adult humans (Kipp et al. 2015), and the maximum tolerable level is 400 μg (United States Department of Agriculture 2012). Considering the daily consumption of 100 g of fresh tuber (composed of 80% water), when applying 0.75 mg kg^{-1} of Se, the content of Se detected in the tubers can contribute to the daily intake of 90 and 20 μg of Se, for selenate and selenite, respectively. However, further research is required involving agronomic biofortification of potatoes with Se, in order to adjust doses to be applied in different soil and environmental conditions, to promote the ideal consumption of Se and to guarantee food security for Brazilians.

The highest accumulation of Se in the shoots was obtained by application of selenite, as observed for the content of the element in the shoot (Fig. 3c). In the tubers, the application of selenate at the dose of 5 mg kg^{-1} increased by 177% the accumulation of the element in relation to the use of selenite in the same dose (Fig. 3d). Comparing to the control, the elevation was 5300% for selenate and 1300% for selenite, respectively.

However, it was expected that the higher content and accumulation of Se in the shoots would be obtained by the application of selenate in relation to selenite, because the selenate present higher mobility inside the plant (Sors et al. 2005; Boldrin et al. 2012), whereas the selenite, when absorbed by the roots is rapidly converted into organic forms and incorporated into organoselenium compounds (White et al. 2004) which have less mobility in the xylem (Li et al. 2008), being more concentrated in the root system rather than transported to the shoot.

Macronutrient and micronutrient contents

For the macronutrients, the interaction between sources and doses affected the contents

of calcium (Ca) and sulfur (S) in the shoots. Nitrogen contents (N) in shoots and S in tubers were influenced only by Se doses (Fig. 4). The other macronutrients were not influenced by the treatments (data not shown).

Nitrogen content in the shoots decreased with the application of Se, independent of the source used, with higher expressiveness at the estimated dose of 3.15 mg kg^{-1} of Se, with a 13% reduction when compared to the control (Fig. 4a). For Ca content in the shoot, applying 5.0 mg kg^{-1} of Se, there was a reduction of 23% and 16%, for selenate and selenite, respectively (Fig. 4b). Previous studies have detected reduction in Ca content in lettuce plants (Smoleń et al. 2014) when the dose of Se was increased in the form of selenate and cucumber for both sources (Hawrylak-Nowak et al. 2015).

Sulfur content in shoots increased by approximately 280% at the dose of 5 mg kg^{-1} of Se in the selenate form, when compared to control; the selenite had not affected for S content in the plants (Fig. 4c). Similar results were obtained for other species such as wheat (Boldrin et al. 2016), cucumber (Hawrylak-Nowak et al. 2015) and rice (Boldrin et al. 2012). This increase may possibly occur because selenate and sulfate resemble chemically and plants absorb and assimilate Se through the same metabolic pathway of S (Sors et al. 2005; Pilon-Smits et al. 2009).

In tubers, the S content increased 2% and 9% at the doses of 0.75 and 1.5 mg kg^{-1} of Se, respectively, relative to the control. On the other hand, the increase in doses of Se caused a reduction in the content of this macronutrient, especially in the highest dose (5.0 mg kg^{-1} of Se), with a reduction of 17% in relation to the control, independent of the source of Se (Fig. 4d). In garlic bulbs, Põldma et al. (2011) detected a reduction in S content after foliar application of high doses of Se (50 μg mL^{-1}) in the form of selenate.

In relation to the micronutrients, the sources of Se affected boron (B) and zinc (Zn) contents in the shoots, and copper (Cu) and Zn contents in the tubers. The selenate source did not differ statistically from the control (Table 1). The other micronutrients were not affected by the treatments (data not shown).

The application of selenite increased the content of B by 9% and reduced by 34% the Zn content in the shoots of the plants; in the tubers, selenite promoted the reduction of 24% and 14% for Cu and Zn, respectively, when compared to selenate. Ramos et al. (2011) observed a reduction in the Zn content in the shoots of lettuce plants (cultivar "Veneranda") when they used selenite, while the selenate application did not differ from the control plants. In brachiaria, Ramos et al. (2012) detected a reduction in Cu and Zn contents in the shoots of the plants when 6 mg kg^{-1} of Se was applied to both sources of Se. The reduction in the content of these micronutrients by the application of Se, especially in the form of selenite, can be attributed to the antagonism between Se and these elements (Feng et al. 2009). In addition, according to Pazurkiewicz-Kocot et al. (2008), the absorption, transport, and accumulation of nutrients in plant cells can be affected due to modifications in the plasma membrane coefficient to the ions.

Physicochemical variables

The pH of the tubers was influenced by the interaction between the sources and doses of Se, while the maturation index (MI) was affected by the doses (Fig. 5). The other physicochemical characteristics (titratable acidity and soluble solids content) were not influenced by the treatments (data not shown).

For the pH of the tubers, only the dose of 5 mg kg^{-1} of Se as selenate promoted

reduction of 2.4% in relation to the control; the selenite reduced the pH in all the applied doses, not being observed differences between them (Fig. 5a).

The MI increased by 40% when applying 3.0 mg kg^{-1} of Se, when compared to the control, the other doses did not differ statistically from the control (Fig. 5b). Zhu et al. (2017) observed delay in fruit maturation of tomato plants grown in nutrient solution with 1 mg L^{-1} of Se as selenate. These changes in tomato fruits occurred due to the increase of the organic acids, as well as the performance of the Se in the reduction of ethylene synthesis, resulting in the increase of organic acids, retarding and/or reducing maturation (Fernandes et al. 2010). This is because acids are used in the process of maturation through respiration and, or their conversion to sugars (Lombardi et al. 2000; Mulyawanti et al. 2010), which results in alterations in the technological quality of the food (Fernandes et al. 2010), and may decrease consumer acceptance of the product.

Biochemical evaluations of tubers

The activity of SOD, CAT, APX, POD and PPO in the tubers were influenced ($p<0.05$) by the application of Se (Fig. 6). The activity of SOD increased with the application of doses of 1.5, 3.0 and 5.0 mg kg^{-1} of Se, regardless of the source (Fig. 6a). For CAT activity, elevation was observed with the application of 1.5 and 3.0 mg kg^{-1} of Se, with reduction in the highest dose (Fig. 6b). APX activity was eleveted as Se doses increased (Fig. 6c).

When Se is provided in low doses, acts as activator of the plant antioxidant system (Djanaguiraman et al. 2005; Ramos et al. 2010, 2012; Saidi et al. 2014; Castillo-Godina et al. 2016). On the other hand, high concentrations may compromise plant metabolism

and, consequently, plant production. This fact is related to the pro-oxidant effect of the element when applied at high concentrations, which may result in damage to cell membranes (Hartikainen et al. 2000). Under stress conditions to the plants, where there is an excess of ROS, SOD is the first enzyme from the antioxidant system to be activated (Mittler 2002), disrupting ROS and producing H_2O_2, which will be neutralized by CAT and APX (Gill and Tutuja 2010). These enzymes act by dismuting H_2O_2 in water and oxygen. In this sense, Ahmad et al. (2016) reported that Se plays an important role in plants, reducing oxidative stress by activating mechanisms of defense against oxidative stress, such as increased SOD, CAT and APX activity.

For the POD activity, there was a reduction of 35% and 38% when applying 5.0 mg kg^{-1} of Se in the form of selenate and selenite, respectively (Fig. 6d). This enzyme exerts influence on the post-harvest quality and, the increase of its activity causes the darkening of tubers and fruits (Campos et al. 1995; Freitas et al. 2008). Therefore, practices that reduce their activity will benefit the maintenance of post-harvest quality of the product. Campos et al. (1995) observed reduction in POD activity in potato tubers when applying Se in the soil, as selenite, reduction that the authors attributed to antioxidant action of Se.

The PPO activity was increased by 16% with the application of selenate (3.0 mg kg^{-1} of Se), for selenite (5.0 mg kg^{-1} of Se) there was a reduction of 21% in activity of this enzyme, in relation to control (Fig. 6e). The effect of PPO on fruits and vegetables in nature results in economic losses and a decrease in nutritional quality, altering the flavor of the products (Campos et al. 1995; Luíz et al. 2007) and increases senescence of vegetables (Freitas et al. 2008). However, under acidic conditions the PPO activity is reduced, which was observed by Pinelli et al. (2005). In the present study, it was found

that the highest dose of Se for both sources resulted in lower tubers pH (Fig. 5a) and lower PPO activity (Fig. 6e).

The contents of H_2O_2 e MDA were influenced by the application of Se (Fig. 7). H_2O_2 was reduced when the doses of 0.75; 1.5 and 3.0 mg kg^{-1} of Se were applied, regardless of the source (Fig. 7a). It is known that the reduction in H_2O_2 concentration in plant cells is related to the performance of the CAT and APX enzymes, since they play an important role in the elimination of these ROS generated in the photorespiration process, or under conditions of oxidative stress (Gill and Tuteja 2010; Noctor et al. 2013). Thus, the increase in the activity of these enzymes caused by the action of Se in low doses may be the explanation for the enhancement of the tolerance of plants to the abiotic stresses, as shown in previous research (Mittler 2002; Alyemeni et al. 2017).

Higher concentration of MDA was identified as the Se dose increased, regardless of the source used (Fig. 7b). This elevation indicates the occurrence of oxidative stress, resulting in damage to cell membranes and production losses, which may justify increase in *gs* and *E* (Fig. 1) as well as the decrease in tuber production (Fig. 2b). This is because MDA is a result of lipid peroxidation and has been used in the identification of damage caused by ROS (Wu et al. 2006). Previous works have shown increased MDA content in plants submitted to high doses of Se (Hartikainen et al. 2000; Ramos et al. 2010, 2012; Hawrylak-Nowak et al. 2015).

In conclusion, the lower doses of Se, as well as the selenate source, are more favorable for biofortification of potato in tropical conditions, especially for the dose 0.75 mg kg^{-1} of Se, since it increases the production of tubers, provides the amount of Se necessary for the daily intake of an adult human, in addition to not compromising the

metabolic processes in plants. However, future field studies are needed to establish the ideal dose for the application of Se in different soils and environmental conditions, especially due to the lack of information about the biofortification of vegetable crops with selenium in such conditions (tropical). Further, anatomical studies of the plants are necessary to ascertain the damages to the vegetal cells caused by the high doses of Se, especially due to the increase of the lipid peroxidation. There is also a need for sensorial tests in order to understand the possible changes in tuber characteristics such as color, taste and cooking time, as well as the acceptance rate of the biofortified products by the consumers.

Acknowledgments

To National Council for Technological Development (CNPq-Brazil), Foundation for Research Support of Minas Gerais (FAPEMIG) and Coordination of Improvement of Higher Level Personnel (CAPES), for financial support and scholarship grant.

Rerefences

Ahmad R, Waraich EA, Nawaz F, Ashraf MY, Khalid M (2016) Selenium (Se) improves drought tolerance in crop plants - a myth or fact? J Sci Food Agric doi: http://dx.doi.org/10.1002/jsfa.7231

Alyemeni MN, Ahanger MA, Wijaya L, Alam P, Bhardwaj R, Ahmad, P (2017) Selenium mitigates cadmium-induced oxidative stress in tomato (*Solanum lycopersicum* L.) plants by modulating chlorophyll fluorescence, osmolyte accumulation, and antioxidant system. Protoplasma 1-11. 10.1007/s00709-017-1162-4

AOAC (2010) Official methods of the Association of the Agricultural Chemists. 18 ed., Arlington: Association of Official Analytical Chemists.

Biemelt S, Keetman U, Albreacht G (1998) Re-aeration following hypoxia or anoxia leads to activation of the antioxidative defense system in roots of wheat seedlings. Plant Physiol 116: 651 –658.

Boldrin PF, Faquin V, Ramos SJ, Guilherme LRG, Bastos CEA, Carvalho GS, Costa ETS (2012) Selenato e selenito na produção e biofortificação agronômica com selênio em arroz. Pesqui Agropecu Bras 47: 831-837.

Boldrin PF, Figueiredo MA, Yang Y, Luo H, Giri S, Hart JJ, Faquin V, Guilherme LRG, Thannhauser TW, Li L (2016) Selenium promoters sulfur accumulation and plant growth in wheat (*Triticum aestivum*). Physiol Plantarum 158: 80–91.

Braun H, Fontes PCR, Finger FL, Busato C, Cecon PR (2010) Carboidratos e matéria seca de tubérculos de cultivares de batata influenciados por doses de nitrogênio. Ciênc Agrotec 34: 285-293.

Campos AD, Freire CJS, Costa DM (1995) Alterações enzimáticas e protéicas nos tubérculos de duas cultivaras de batata (*Solanum tuberosum* L.) em decorrência da aplicação de selênio no solo. Pesq Agropec Bras 30: 903-907.

Castillo-Godina RG, Foroughbakhch-Pournavab R, Benavides-Mendoza A (2016) Effect of selenium on elemental concentration and antioxidant enzymatic activity of tomato plants. J. Agr. Sci. Tech 18: 233-244.

Djanaguiraman M, Devi DD, Shanker AK, Sheeba JA, Bangarusamy U (2005) Selenium: an antioxidative protectant in soybean during senescence. Plant Soil 272: 77-86.

Embrapa, Empresa Brasileira de Pesquisa Agropecuária (1997) Manual de métodos de análise de solo. Rio de Janeiro, 212p. (Documentos 1).

FAO (2013) Available in: http://faostat.fao.org/faostat. Acess: dec. 2016.

Feng R, Wei C, Tu S, Wu F (2009) Effects of Se on the uptake of essential elements in Pteris vittata L. Plant Soil 325: 123–132.

Fernandes AM, Soratto RP, Evangelista RM, Nardin I (2010) Qualidade físico-química e de fritura de tubérculos de cultivares de batata na safra de inverno. Hortic Bras 28: 299-304.

Freitas AA, Francelin MF, Hirata GF, Clemente E, Schmidt FL (2008) Atividades das enzimas peroxidase (POD) e polifenoloxidase (PPO) nas uvas das cultivares benitaka e rubi e em seus sucos e geleias. Ciênc. Tecnol. Aliment 28: 172-177.

Giannopolitis CN, Ries SK (1977) Superoxide dismutases: I. Occurrence in higher plants. Plant Physiol 59: 309-314.

Gill SS, Tuteja N (2010) Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol *Biochem* 48: 909-930.

Hartikainen H, Xue T, Piironen V (2000) Selenium as an anti-oxidant and pro-oxidant in ryegrass. Plant Soil 225: 193–200.

Havir EA, McHale NA (1987) Biochemical and developmental characterization of multiple forms of catalase in tobacco leaves. Plant Physiol 84: 450–455.

Hawrylak-Nowak B, Matraszek R, Pogorzelec M (2015) The dual effects of two inorganic selenium forms on the growth, selected physiological parameters and macronutrients accumulation in cucumber plants. Acta Physiol Plant 37: 1–13.

Ježek P, Hlušek J, Lošák T, Jůzl M, Elzner P, Kráčmar S, Buňka F, Martensson A (2011)

Effect of foliar application of selenium on the content of selected amino acids in potato tubers (*Solanum tuberosum* L.). Plant soil Environ 57: 315–320.

Kipp AP, Strohm D, Brigelius-Flohé R, Schomburg L, Bechthold A, Leschik-Bonnet E, Heseker H (2015) Revised reference values for selenium intake. J. Trace Elem. Med. Biol 32: 195–199.

Li HF, McGrath SP, Zhao FJ (2008) Selenium uptake, translocation and speciation in wheat supplied with selenate or selenite. New Phytol 178: 92–102.

Lombardi SRB, Moraes DM, Camelatto D (2000) Avaliação do crescimento e da maturação pós-colheita de pêras da cultivar Shinsseiki. Pesqui Agropecu Bras 35: 2399-2405.

Luíz RC, Hirata TAM, Clemente E (2007) Cinética de inativação da polifenoloxidase e peroxidase de abacate (*Persea americana* Mill.). Ciênc Agrotec 31: 1766-1773.

Malavolta, E. (1980) Elementos de nutrição mineral de plantas. São Paulo: Agronômica Ceres. 251p.

Malavolta E, Vitti GC, Oliveira SA (1997) Avaliação do estado nutricional das plantas: princípios e aplicações. 2nd. ed. Piracicaba: Potafos.

Matsumo H, Uritani I (1972) Physiological behavior of peroxidase enzymes in sweet potato root tissue injured by cutting or black root. Plant Cell Physiol 13: 1.091-1.101.

Mittler R (2002) Oxidative stress, antioxidants and stress tolerance. Trends and Plant Science 7: 405–410.

Montillet JL, Chamnogpol S, Rustérucci C, Dat J, Van de Cotte B, Agnel JP, Triantaphylides C (2005) Fatty acid hypersensitive and H_2O_2 in the execution of hypersensitive cell death in tobacco leaves. Plant Physiol 138: 1516–1526.

Mulyawanti I, Dewandari KT, Yulianingsih (2010) Effects of freezing and storage periods on characteristics of frozen sliced *Arumanis mango*. Indonesian Journal of Agriculture 3: 32-38.

Nawaz F, Ahmad R, Ashraf MY, Waraich EA, Khan SZ (2015) Effect of selenium foliar spray on physiological and biochemical processes and chemical constituents of wheat under drought stress. Ecotox Environ Safe 113: 191–200.

Noctor G, Mhamdi A, Queval G, Foyer C (2013) Regulating the redox gatekeeper: vacuolar sequestration puts glutathione disulfide in its place. Plant Physiol 163: 665-617.

Pazurkiewicz-Kocot K, Kita A, Pietruszka M (2008) Effect of selenium on magnesium, iron, manganese, copper, and zinc accumulation in corn treated by indole3-acetic acid. Commun Soil Sci Plan 39: 2303–2318.

Pilon-Smits EAH, Quinn CF, Tapken W, Malagoli M, Schiavon M (2009) Physiological functions of beneficial elements. Curr Opin Plant Biol 12: 267-274.

Pinelli LLO, Moretti CL, Almeida GC, Nascimento ABG, Onuki ACA (2005) Associação de atmosfera modificada e antioxidantes reduz o escurecimento de batatas 'Ágata' minimamente processadas. Hortic Bras 23: 993-999.

Põldma P, Tõnutare T, Viitak A, Luik A, Moor U (2011) Effect of Selenium Treatment on Mineral Nutrition, Bulb Size, and Antioxidant Properties of Garlic (*Allium sativum* L.). J Agr Food Chem 59: 5498–5503.

Puccinelli M, Malorgo F, Pezzarossa B (2017a) Selenium enrichment of horticultural crops. Molecules 22: 933.

Puccinelli M, Malorgioa F, Rosellinib I, Pezzarossab B (2017b) Uptake and partitioning of selenium in basil (*Ocimum basilicum* L.) plants grown in hydroponics. Sci Hortic-

Amsterdam 225: 271–276.

R Core Team. R: A language and environment for statistical computing (2015) R Foundation for Statistical Computing, Vienna, Austria. Available in: https://www.R-project.org (Acess: Jun. 2017).

Ramos SJ, Faquin V, Guilherme LRG, Castro EM, Avila FW, Carvalho GS, Bastos CEA, Oliveira C (2010) Selenium biofortification and antioxidant activity in lettuce plants fed with selenate and selenite. Plant Soil and Environ 56: 584-588.

Ramos SJ, Rutzke MA, Haynes RJ, Faquin V, Guilherme LRG, Li L (2011) Selenium accumulation in lettuce germplasm. Planta 233: 649-660.

Ramos SJ, Ávila FW, Boldrin PF, Pereira FJ, Castro EM, Faquin V, Reis AR, Guilherme LRG (2012) Response of brachiaria grass to selenium forms applied in a tropical soil. Plant Soil Environ 58: 521–527.

Rayman MP (2012). Selenium and human health. Lancet 379: 1256–1268.

Rosen BP, Liu Z (2009) Transport pathways for arsenic and selenium: a minireview. Environ Int 35: 512–515.

Saidi I, Chtourou, Y, Djebali W (2014) Selenium alleviates cadmium toxicity by preventing oxidative stress in sunflower (*Helianthus annuus*) Seedlings. J. Plant Physiol 171: 85-91.

Schiavon M, Lima LW, Jiang Y, Hawkesford MJ (2017) Effects of Selenium on Plant Metabolism and Implications for Crops and Consumers. In: Pilon-Smits EAH, et al. Selenium in plants, Plant Ecophysiology**,** 11, 2017.

Scott AJ, Knott M (1974) A cluster analysis method for grouping means in the analysis of variance. Biometrics 30: 507-512.

Smoleń S, Kowalska I, Sady W (2014) Assessment of biofortification With Iodine and Selenium of Lettuce Cultivated in the NFT Hydroponic System. Sci Hortic-Amsterdam 166: 9-16.

Sors TG, Ellis DR, Salt DE (2005) Selenium uptake, translocation, assimilation and metabolic fate in plants. Photosynth Res 86: 373-389.

Teisson C (1979) Lê brunissement interne de I'ananás. I-Historique. II-Material et methodos. Fruits 34: 245-281.

Turakainen M, Hartikainen H, Seppänen MM (2004) Effects of selenium treatments on potato (*Solanum tuberosum* L.) growth and concentrations of soluble sugars and starch. J Agric Food Chem 52: 5378-5382.

USDA (2012) USDA, National Nutrient Database for Standard Reference*, Release 25*. Nutrient Data Laboratory Home Page. U.S. Department of Agriculture, Agricultural Research Service. Retrieved from: http://www.ars.usda.gov/ research/publications/Publications.htm?seq_no_115=285841

United States Environmental Protection Agency – USEPA (1998) Method 3051a – Microwave assisted acid digestion of sediments, sludges, soils, and oils. Revision 1 Fev 2007. 30p. Available in: ‹http:// www.epa.gov/epawaste/hazard/testmethods/sw846/pdfs/ 3051a.pdf›. Acess: jun. 2017.

Velikova V, Yordanov I, Edreva A (2000) Oxidative stress and some antioxidant systems in acid rain-treated bean plants protective role of exogenous polyamines. Plant Sci. 151: 59 –66.

White PJ, Brown PH (2010) Plant nutrition for sustainable development and global

health. Ann Bot-London 105: 1073-1080.

Wu FB, Dong J, Jia G, Zheng S, Zhang G (2006) Genotypic difference in the responses of seedling growth and Cd toxicity in rice (*Oryza sativa* L.). Agr Sci China 5: 68-76.

Zhang Y, Pan G, Chen J, Hu Q (2003) Uptake and transport of selenite and selenate by soybean seedlings of two genotypes. Plant Soil 253: 437-443.

Zhu Z, Chen Y, Shi G, Zhang X (2017) Selenium delays tomato fruit ripening by inhibiting ethylene biosynthesis and enhancing the antioxidant defense system. Food Chem 219: 179-184.

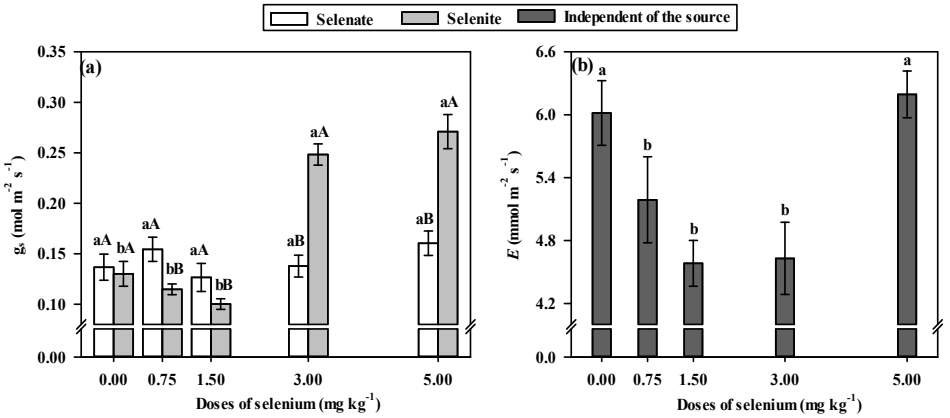

Fig. 1 Stomatal conductance-g_s as a function of sources and doses of Se (a) and foliar transpiration-E as a function of Se doses (b) in potato plants grown on tropical soil. Equal letters, lowercase comparing the doses for each source and uppercase comparing the sources within each dose do not differ from each other (Scott-Knott, $p \leq 0.05$). Vertical bar indicates the standard error of the mean (n = 4).

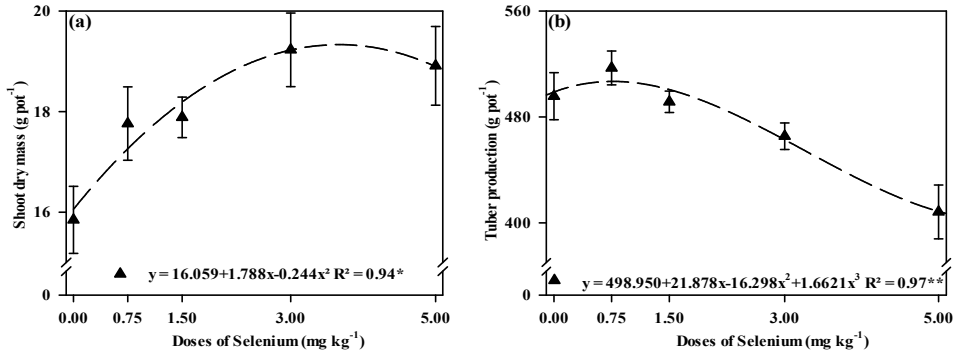

Fig. 2 Shoot biomass (a) and tuber production (b) of potato plants as a function of Se doses. Vertical bar indicates the standard error of the mean (n = 4).

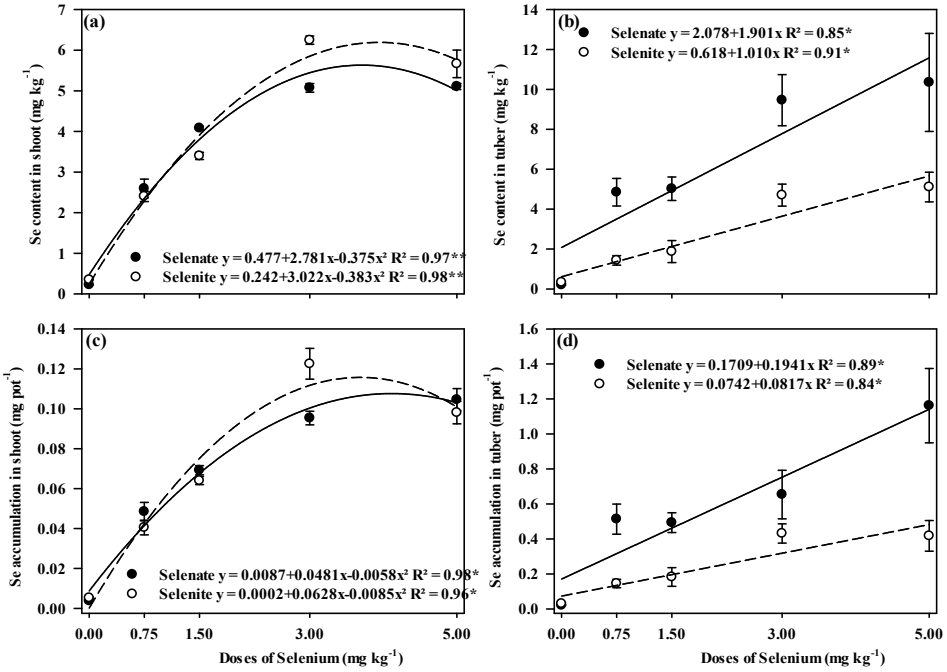

Fig. 3 Content and accumulation of Se in shoot (a, c) and tubers (b, d) of potato plants as a function of sources and doses of Se. Vertical bar indicates the standard error of the mean (n = 4).

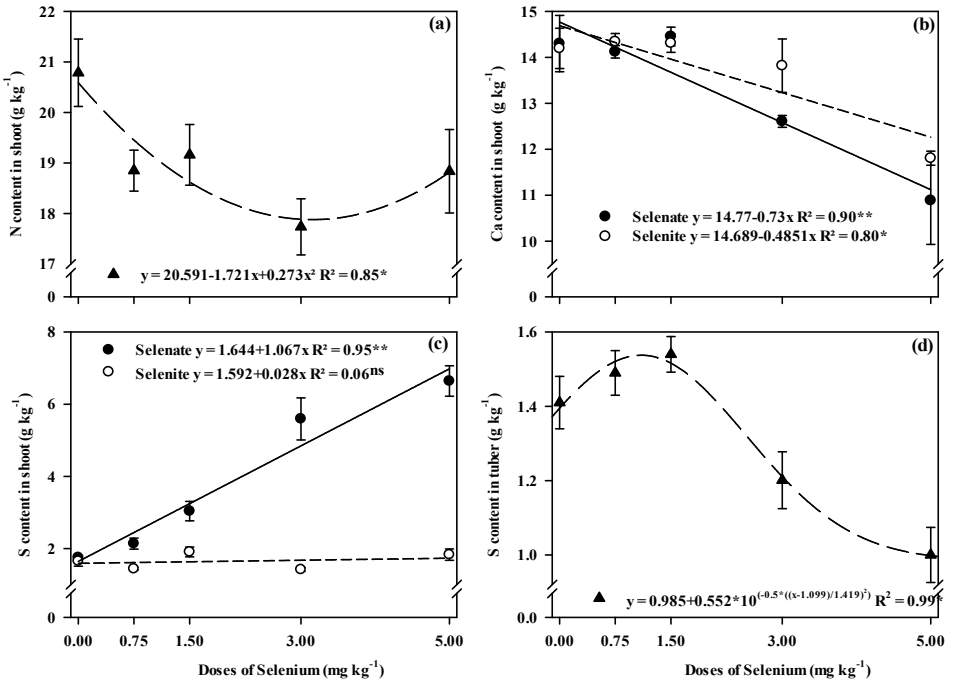

Fig. 4 N (a), Ca (b) and S (c) contents in the shoots and S (d) content in potato tubers as a function of sources and doses of Se. Vertical bar indicates the standard error of the mean (n = 4).

Table 1 Boron (B) and zinc (Zn) contents in shoot and copper (Cu) and Zn contents in tubers of potato as a function of Se sources.

Sources	Shoot		Tuber	
	B	Zn	Cu	Zn
	---------- mg kg^{-1} ----------			
Control	53.33 a	63.05 a	4.39 a	30.69 a
Selenate	46.64 b	68.22 a	4.56 a	28.24 a
Selenite	52.11 a	42.54 b	3.56 b	25.13 b

Means followed by equal letters in the column do not differ from each other (Scott-Knott, $p \leq 0.05$).

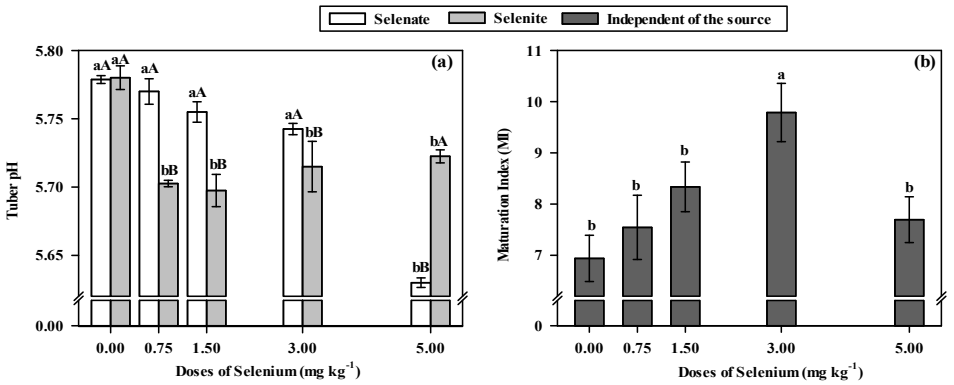

Fig. 5 pH of the potato tubers as a function of doses and sources of Se (a) and maturation index of the tubers as a function of Se doses (b). Equal letters, lowercase comparing the doses for each source and uppercase comparing the sources within each dose do not differ from each other (Scott-Knott, $p \leq 0.05$). Vertical bar indicates the standard error of the mean (n = 4).

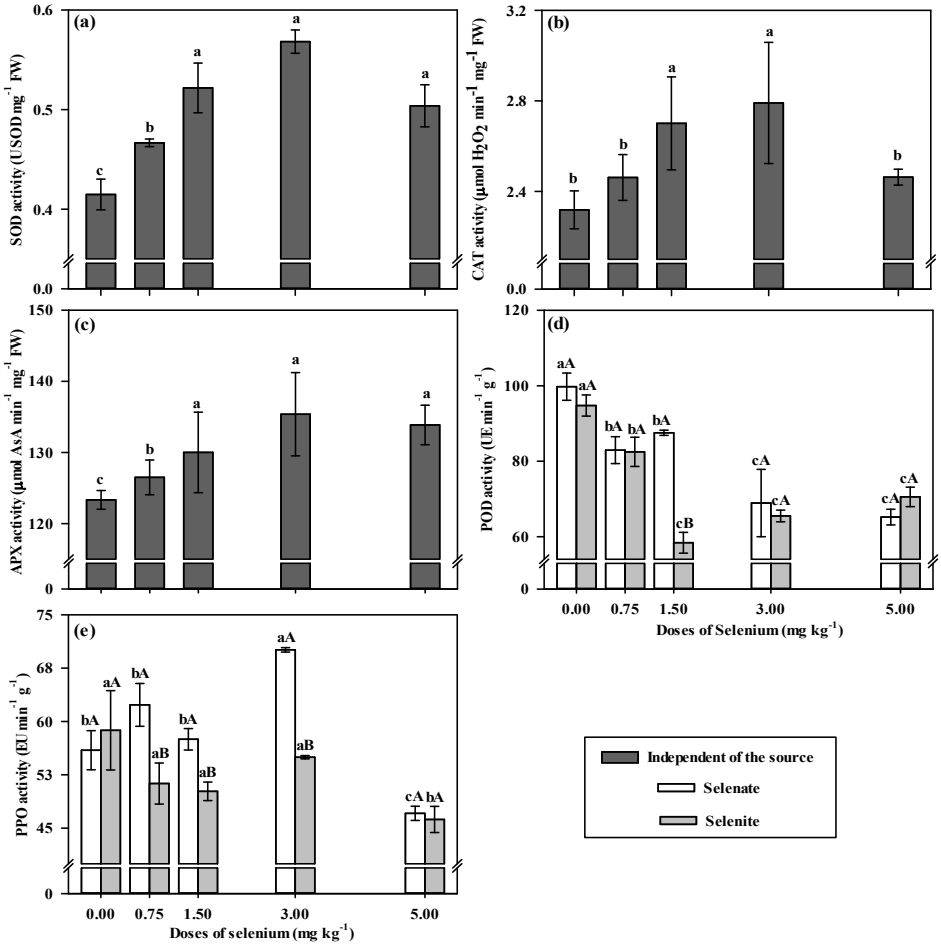

Fig. 6 Effect of Se application on enzymatic activity in potato tubers. Activity of superoxide dismutase (SOD) (a), catalase (CAT) (b), ascorbate peroxidase (APX) (c), polyphenoloxidase (PPO) (d) and peroxidase (POD) (e). Equal letters, lowercase comparing the doses for each source and uppercase comparing the sources within each dose do not differ from each other (Scott-Knott, $p \leq 0.05$). Vertical bar indicates the standard error of the mean (n = 4).

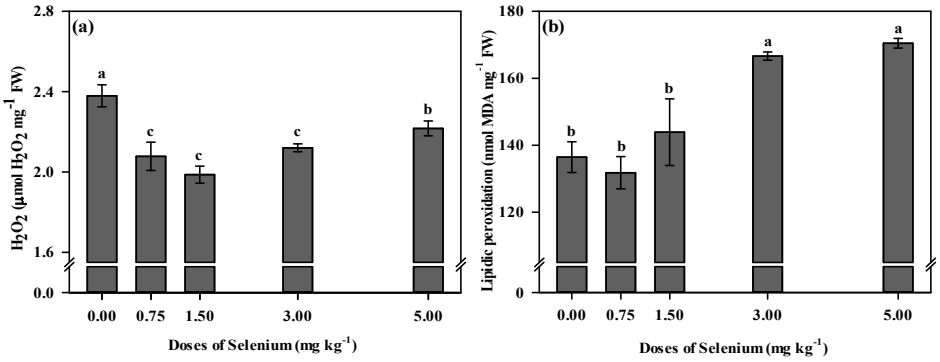

Fig. 7 Effect of Se in the concentration of hydrogen peroxide (a) and MDA (lipidic peroxidation) (b) in potato tubers. Equal letters, lowercase comparing the doses, do not differ from each other (Scott-Knott, $p \leq 0.05$). Vertical bar indicates the standard error of the mean (n = 4).

ARTIGO 2

Agronomic biofortification of potato with selenium applied via foliar in tropical conditions

(Artigo submetido ao periódico *Scientific Reports*)

Agronomic biofortification of potato with selenium applied via foliar in tropical conditions

Vanuze C. de Oliveira[1,2], Valdemar Faquin[2], Joelma Pereira[3], Luiz R. G. Guilherme[2] & Jéssica C. Teodoro[4]

Abstract Selenium (Se) is a micronutrient to the human and animal body, acting on the antioxidant system. Even though Se is not essential for plants, low doses can be beneficial. This study aimed to evaluate the effect of foliar application of Se from different sources and concentrations in plants of potato from the cultivar Ágata. Plants were grown in pots containing red-yellow Latosol. In the tuberization period two Se sources were applied via foliar (sodium selenate or selenite) in five concentrations (0; 25; 50; 75 and 100 μmol L^{-1} of Se), applying 200 mL per plant. Physiological, agronomic and nutritional traits were evaluated along with physico-chemical and biochemical analysis in tubers. The results showed that the application of Se, regardless of the source, increases the content of the element in the tubers; the application of selenate raises the internal concentration of CO_2 and stomatal conductance of the plants, as well the content of B in the shoot and K, S, Fe, SS and activity of the SOD in tubers; besides decreases H_2O_2 concentration and lipid peroxidation in tubers. Despite the beneficial effects of Se in potato plants, it is still necessary to carry out sensorial tests to

[1] (✉) Vanuze Costa de Oliveira
vanuze.costa@gmail.com, Orcid: 0000-0001-8979-5484
[2] Departament of Soil Science, Federal University of Lavras, Lavras - MG, Brazil
[3] Departament of Food Science, Federal University of Lavras, Lavras - MG, Brazil
[4] Department of Biology, Federal University of Lavras, Lavras - MG, Brazil

identify changes in color, cooking time and flavor of tubers, and the more importantly, to identify the acceptance index of biofortified foods by the consumers.

Keywords *Solanum tuberosum* L., selenate, selenite, physical-chemical characteristics, antioxidant activity.

Introduction

Selenium (Se) is a micronutrient for humans and animals because it is part of several selenoproteins that are associated with the body's antioxidant system, as well as being related to the immune and reproductive system[1]. The daily requirement of Se to be ingested varies with the age of the human, being 10 μg for infants up to 4 months old and 70 μg for adult humans[2]. Despite being required at low daily doses, a large part of the world population is deficient in this nutrient, resulting in serious health problems, such as the Keshan's disease[3], ocular problems[4] and reproductive problems[5], in addition to various cancers[6].

Nutrient deficiency can be contoured through a diversified diet, the use of dietary supplements, industrial fortification and the consumption of biofortified foods obtained through biofortification, which is defined as a technique that increases the content of an element in edible parts of plants. Agronomic biofortification can be carried out through the nutrient insertion by fertilization of the crops via soil, leaves or hydroponic cultivation. Several studies have shown the success of biofortification with Se[7-10]. Finland adopts this technique in agricultural production, resulting in an increase in the content of this element in the blood plasma of the population, once deficient in this micronutrient[11].

In plants, Se is found mostly in organic form[7], which is more easily absorbed and metabolized by the animal and human organism[12]. Thus, Se biofortification in foods allows inserting the element in the food chain, having the plants as allies for acting in the control of excessive and/or accidental intake that might occur in humans and animals that use food supplements with Se.

The Se is not essential for plants, however, several studies have shown its beneficial effect on the plant metabolism[8,10,13,14], since the element supplied in low concentrations increases the activity of antioxidant system enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and decreases lipid peroxidation.

Although benefic, most plant species are sensitive to high doses of Se, which requires attention when establishing the amount of the element to be applied in plants, because high doses can compromise metabolism and, consequently, plant production due to its pro-oxidant effect[15]. Another important factor for the success of biofortification is the source to be used, since the inorganic sources (selenate and selenite) have different behaviors inside the plants, and it has been demonstrated that selenate is more efficient for the biofortification of several plant species such as lettuce[8], carrot[7], rice[9], wheat[16].

Besides the already mentioned factors, for the biofortification success the target culture must present high consumption by the population. In this sense, potato (*Solanum tuberosum* L.) stands out as one of the five most cultivated and consumed crops in the world[17], besides having several elements and vitamins essential for the human organism. In addition, previous research has shown that the supply of the element increased its content in potato tubers and improved physiological characteristics of plants[18,19].

Although research has shown the success of the agronomic biofortification of several

agricultural species, only few studied have been conducted in tropical soil conditions, concerning the biofortification of vegetable crops with Se and of the potato biofortification in a tropical environment no research were reported in the literature, showing the need for more studies. In this context, the objective of this study was to evaluate the effect of foliar application of Se inorganic sources and different concentrations in potato cultivation under soil conditions.

Materials and methods

Characterization of the experiment, experimental design and application of Se

The experiment was conducted under greenhouse with potato (*Solanum tuberosum* L.), cultivar Ágata, at the Federal University of Lavras, Lavras-MG, Southeastern Brazil (21 ° 22'62 "S, 44 ° 97'94" W and altitude of 918 m) from December 2016 to April 2017. The minimum and maximum temperatures and the relative humidity of the air throughout the crop cycle were, on average, 20 and 33 °C and 67%, respectively.

Seed potatoes with a mean weight of 50 g were seeded in pots containing 7 kg of dystrophic Red-Yellow Latosol collected in the 0-20 cm layer, with the following chemical and physical characteristics: pH in water: 4.8; P-Mehlich: 1.1 mg dm^{-3}; P-rem: 26.6 mg dm^{-3}; K: 32 mg dm^{-3}; Ca: 0.3 $cmol_c$ dm^{-3}; Mg: 0.1 $cmol_c$ dm^{-3}; Al: 0.6 $cmol_c$ dm^{-3}; H+Al: 4.5 $cmol_c$ dm^{-3}; SB: 0.5 $cmol_c$ dm^{-3}; SOM: 16 g kg^{-1}; V: 9.6%; t: 1.1 $cmol_c$ dm^{-3}; T: 5.0 $cmol_c$ dm^{-3}; m: 55.5%; S: 9.9 mg dm^{-3}; Zn: 0.5 mg dm^{-3}; B: 0.2 mg dm^{-3}; Fe: 41.6 mg dm^{-3}; Mn: 4.1 mg dm^{-3}; Cu: 0.5 mg dm^{-3}. Sand 740 g kg^{-1}; silt: 30 g kg^{-1} and clay: 230 g kg^{-1} and natural content of Se of 0.065 mg dm^{-3}.

Liming was performed based on the chemical analysis of the soil, raising the base

saturation to 60% using dolomitic limestone (CaO: 37%, MgO: 15% and PRNT: 85%). The soil was incubated for 30 days at about 60% moisture content of the total pore volume (VTP). Planting and cover fertilization were performed done using 380 mg dm^{-3} of N and 400 mg dm^{-3} of K (performed in four applications); 350 mg dm^{-3} of P; 50 mg dm^{-3} of S; 0.5 mg dm^{-3} of B; 1.5 mg dm^{-3} of Cu; 5 mg dm^{-3} of Zn and 0.1 mg dm^{-3} of Mo.

A completely randomized 2x5 factorial design was used with two sources of Se (sodium selenate-Na_2SeO_4 and sodium selenite-$Na_2SeO_3.5H_2O$ [Sigma-Aldrich, Saint Louis, USA]) and five concentrations of Se (0, 25, 50, 75 and 100 μmol L^{-1}), applying 200 mL per plant, with four replications, totaling 40 plots. Each experimental unit consisted of a pot containing a plant. The application of Se was carried out via foliar in the stage of plant tuberization using a CO_2 pressurized sprayer with constant pressure of 2.8 kgf cm^{-2}; the solution was evenly distributed in all leaves. During the crop cycle the plants were irrigated with deionized water, maintaining the soil moisture close to the field capacity.

Gaseous exchanges, production of shoot dry mass and production of fresh tubers

Measurements of gas exchange were performed 80 days after sowing and 20 days after the application of Se, and IRGA (LI-6400XT, LI-COR, Nebraska, USA) was used for this purpose. The determined variables were: carbon assimilatory rate (*A*-μmol CO_2 m^{-2} s^{-1}), transpiratory rate (*E*-mmol H_2O m^{-2} s^{-1}), stomatal conductance (*gs*-mol H_2O m^{-2} s^{-1}) and internal concentration of CO_2 (*Ci*-mmol CO_2 m^{-2} s^{-1}). The readings were performed between 9:30 a.m. and 11:50 a.m., based on the third fully expanded leaf and exposed to sunlight on a cloudy day with average air temperature and relative humidity of 28 °C

and 60%, respectively.

After 105 days of sowing, irrigation was suspended for desiccation of the branches; after complete drying, plants were harvested by separating the shoot and tubers. The aerial part was dried in a drying oven with forced air circulation at 70 °C until constant weight, obtaining the dry mass of the aerial part, and the fresh tubers were washed and weighed to obtain the production.

Determination of Se, macronutrients and micronutrients

For the determination of Se, dry shoot samples and tubers were milled in a Willey mill with 1.0 mm (40 mesh) sieves. For the analysis of the contents of Se and nutrients, five tubers of each experimental plot were partitioned and dried in a greenhouse with forced air circulation 70 °C until constant weight. Then 0.5 g of each sample was digested in 5 mL of nitric acid in microwave oven (CEM, Mars Model 5CEM Corporation, Matthews, USA), according to USEPA 3051A[20]. After cooling, 5 mL of deionized water was added to the extract. For analytical control, a sample was placed with standard reference material (White Clover - BCR 402, Institute for Reference Materials and Measurements, Geel, Belgium) with Se content of 6.7 mg kg^{-1}. The mean recovery for Se in this material was 92% (n = 4). The reading was performed by atomic absorption spectroscopy in a graphite furnace (Perkin Elmer, model AA-analyst 800, Midland, Canada).

The plant tissues were digestes and the solutions were analyzed for the determination of macro and micronutrient contents[21]. The contents of Se, macronutrients and micronutrients in shoot and tubers were expressed in dry weight.

Physical-chemical and biochemical characteristics of tubers

From each experimental plot, five tubers were separated and stored in liquid nitrogen at -80 °C. The antioxidant activities of SOD, CAT, APX, peroxidase (POD), polyphenoloxidase (PPO), concentration of hydrogen peroxide (H_2O_2) and MDA (lipid peroxidation) were determined.

The pH of the tubers was determined using 5 g of shredded tubers in 10 mL of distilled water and reading was realized in a digital potentiometer (Digimed, model DM-20)[22]. Soluble solids (SS) were quantified by maceration of 5 g of the tubers and then two drops of the liquid were placed in the prism of the electronic refractometer (Atago, model PR100)[23]. To determine the titratable acidity (TA), 5 g of the tubers pulp were ground with 10 mL of distilled water, followed by filtration and the titration with 0.1 mol L^{-1} sodium hydroxide solution, using phenolphthalein[22] as the indicator. From the SS/AT ratio, the MI was obtained[22].

The extraction of the enzymes for the determination of POD and PPO activity was performed[23]. A Femto spectrophotometer, model 600 Plμs was used to read the extracts, being carried at 470 nm and 395 nm of absorbance for a POD[24] and PPO[25], respectively.

For the determination of SOD, CAT and APX activities the enzymes were extracted[26], in which 0.2 g of tubers were macerated in liquid nitrogen and 22 mg of polyvinylpolypyrrolidone (PVPP) and 1.5 mL of the buffer were added with 375 μL of 100 mmol L^{-1} potassium phosphate (pH 7.8), 15 μL EDTA (0.1 mmol L^{-1}), 75 μL ascorbic acid (10 mmol L^{-1}) and 1035 μL water. The samples were then centrifuged at 13000 g for 10 minutes at 4 °C; after this process the supernatant was collected and stored at -80 °C.

The determination of SOD was done by adding 5 µL of the enzyme extract and 5 µL of water to 171 µL of the compound: 50 mmol L^{-1} potassium phosphate (pH 7.8), methionine (14 mmol L^{-1}), EDTA (0.1 µmol L^{-1}), NBT (75 µmol L^{-1}) and riboflavin (2 µmol L^{-1}). The medium remained illuminated by a 15W fluorescent lamp for 7 minutes[27]. The readings were performed at 560 nm using a spectrophotometer (Epoch-Bioteck-Elisa).

Catalase (CAT) activity was determined by adding 4.5 µL of the enzyme extract with 4.5 µL of water to 171 µL of the buffer composed of 90 µL of 100 mmol L^{-1} potassium phosphate (pH 7, 0), 9 µL of hydrogen peroxide (250 mmol L^{-1}) and 72 µL of distilled water; samples were then incubated at 30 °C[28]. The reading was performed at 240 nm, using a spectrophotometer (Epoch-Bioteck-Elisa).

For the APX activity determination, 4.5 µL of the enzyme extract and 4.5 µL of water were added to 171 µL of the buffer composed of 90 µL of 200 mmol L^{-1} potassium phosphate (pH 7.0), 9 µL of ascorbic acid (10 mmol L^{-1}), 9 µL of hydrogen peroxide (2 mmol L^{-1}) and 63 µL of distilled water. The reading was performed at 290 nm[29].

For the quantification of hydrogen peroxide (H_2O_2) and concentration of MDA (lipid peroxidation), the extract was obtained by maceration of 0.2 g of tubers in liquid nitrogen and 22 mg of PVPP, followed by the addition of 1.5 mL of trichloroacetic acid (0.1%). Posteriorly, the mixture was centrifuged at 12,000 *g* for 15 minutes at 4 °C for supernatant collection.

Contents of H_2O_2 were quantified by measuring the absorbance at 390 nm in spectrophotometer (Epoch-Bioteck-Elisa) in a reaction composed of 45 µL of the supernatant collected after centrifugation, 45 µL of potassium phosphate (10 mmol L^{-1})

and 90 µL of potassium iodide (1 mol L^{-1})[30]. The H_2O_2 quantification was done by means of the standard curve with known H_2O_2 concentrations.

Lipid peroxidation was determined[31]. For this, aliquots of the extract (125 µL) were added to the reaction medium containing triobarbituric acid (0.5%) and trichloroacetic acid (10%) and then incubated at 95 °C for 30 minutes until the reaction was stopped by fast cooling using ice. The readings were carried out in a spectrophotometer (Epoch-Bioteck-Elisa) at 535 and 600 nm and the results were presented in mmol of malondialdehyde (MDA) per milligram of fresh mass.

Statistical analysis

The data were submitted to analysis of variance (Anova) and the significance was verified by the F test. The Scott-knott test[32] was applied at 5% significance along with regression analysis. The software R[33] was used for statistical analysis and the SigmaPlot 12.5 software (Systat Software, Inc., San Jose, CA - USA) for graphing.

Results and discussion

Production of aerial part dry mass, fresh tubers and gas exchange

The doses and sources of Se applied on leaves did not affect the production of aerial part dry mass and fresh potato tubers (data not shown).

Among the variables analyzed for the gas exchange, effects on the internal carbon concentration (*Ci*), stomatal conductance (g_s) and photosynthetic rate (*A*) were observed, with *Ci* and g_s being influenced by the sources of Se and *A* by Se concentrations (Fig. 1). The treatments did not affect leaf transpiration (*E*) (data not shown).

The application of selenate did not differ of the control provideing the highest values

of *Ci*, with increased of 14% in relation to selenite (Fig. 1a); to the g_s, the selenate increased in 7% when compared to selenite that did not differ of the control (Fig. 1b). The photosynthetic rate (*A*) increased with the application of Se at concentrations of 50, 75 and 100 μmol L^{-1} relative to the control (Fig. 1c). Increase in *A*, g_s and *E* was found in wheat plants when foliar application of 40 mg L^{-1} of Se was applied as selenate[34]. Previous studies have demonstrated the beneficial effect of the low dose application of Se on plant gas exchange, in which the authors related to a possible reduction in chlorophyll degradation, resulting in increased plant biomass[35].

Selenium has a hormesis effect on plants, which means that at low doses the element provides benefits to the plant metabolism and high concentrations cause phytotoxicity due to its performance as a pro-oxidant[15,36,37] causing elevation of H_2O_2 concentration and lipid peroxidation of cells[8], resulting in damage to cell membranes and reduction of plant growth. When undergoing some stress process, plants develop adaptation mechanisms, such as the increase in the stomatal density and the reduction of the stomata size, to overcome or reduce this stressful condition[38-40]. In this sense, the increase of *A* detected in potato plants in the highest doses of Se can be attributed to possible damages in the guard cells due the excess of reactive oxygen species (ROS) and lipid peroxidation and, as a way of adaptation to the stress caused by Se, there might have been changes in leaf anatomy, such as increased density and reduction of stomata size, resulting in a higher photosynthetic rate[41].

Most agricultural crops are sensitive to high concentrations of Se in tissue, but the sensitivity varies among plant species, requiring care in the establishment of the doses to be applied[42]. For most plant species, the increase in the photosynthetic rate is directly

related to the increase in the synthesis of photosynthetic products and higher dry mass production[43], but it did not occur with potato plants, since there was no effect of the foliar application of Se on the production of aerial part dry mass and fresh tubers.

In *Brassica* species[44] was not observed effect of leaf application of Se (30 g ha^{-1}) on plants yield. The same results were also obtained in rice[9] with foliar application of solutions with 50 μmol L^{-1} de Se. According to the researchers, these results may be related to the shorter contact time of the element with the plants, since in the period when the element was supplied the formation of dry mass was already established[9].

Content and accumulation of Se

The content and accumulation of Se in the aerial part were influenced ($p < 0.05$) by the interaction between the sources and the concentrations of Se, presenting a linear increase with the increase of Se concentration in the solution of foliar application (Fig. 2).

With the exception of the concentration 25 μmol L^{-1} of Se, the highest levels and accumulation of Se in the aerial part were obtained with the application of selenite in expressive values when compared with the control and selenate (Fig. 2a, c). These results corroborate the studies with ryegrass[45], rice[10] and lettuce[46].

When absorbed by the roots, the selenite applied to soil is rapidly converted to organic forms[47] with less translocation inside the plant by the xylem, which possibly also occurs by the phloem when the application is via leaf, where Se is more concentrated. This fact would justify the higher content and accumulation of Se in the aerial part of the plants when the element is supplied via foliar in selenite form.

In the tubers, both the content and the accumulation of Se were affected only by the

concentration of the element applied in the solution (Fig. 2b, d), and with linear and significant increases when compared to the control. However, it was expected that the content of Se in the tubers was higher when applied as selenate, as was already observed in previous research with rice[9] and potato[48]. Selenate is more easily transported through the phloem when compared to the xylem[9,48]. However, the possible explanation for the lack of effect of the sources on the content of Se in the tubers might be related to the period of application of the element, since in the previous research, up to 30 days after the application of the Se in the potato, there was no statistical difference for the Se content by the application of selenate or selenite and only after 60 days of application it was observed a statistical difference for the sources of the element[48]. Higher Se content was found in carrot roots[49] when applying 100 μg mL^{-1} of Se via foliar application in the form of selenate in relation to selenite; however, when using the dose of 10 μg mL^{-1} of Se, there was no statistical difference between sources.

For biofortification purposes, it is of great importance to consider the content of Se in edible parts of the vegetable, which in the case of potatoes are the tubers. Thus, the results showed that the application of Se via foliar is an efficient way of enriching the potato tubers with this element.

The daily dose of Se to be ingested by adult humans is 70 μg^2 and the maximum tolerable level is 400 μg[50]. By correlating this information with the results obtained by the application of 100 μM of Se, it was verified that the content of Se detected in the tubers (3.45 mg kg^{-1} of Se) can contribute with the daily intake of 69 μg Se (considering the consumption of 100 g of fresh tubers and that these are composed of 80% of water). Although it does not meet all needed Se daily intake, it is worth noting that nutritional

needs should not be met by eating only one food, but by means of diversification in the diet. Thus, the consumption of this biofortified potato presents as an alternative for the increase of the intake of Se, contributing to reduce the deficiency of this micronutrient in the human organism. However, further studies involving the application of Se in potato are necessary, aiming to adapt doses to be used in different geographic regions and environmental conditions and, thus, promoting the ideal intake of Se to ensure food safety for humans.

Macronutrient and micronutrient contents

Magnesium (Mg) content in shoot and calcium (Ca), sulfur (S), potassium (K) in tubers, as well the micronutrients boron (B) in shoot and iron (Fe) in tubers were influenced by the application of Se (Fig. 3). The other macronutrients and micronutrients were not affected by the treatments (data not shown).

The Mg content in the aerial part decreased as the Se concentrations increased, regardless of the used source (Fig. 3a). However, Ca content in tubers was influenced by the interaction between the sources and concentrations of Se. The application of higher concentration of Se in the form of selenite reduced Ca content in 24% tubers (Fig. 3b) when compared to the control. The application of selenate reduced Ca content in the tubers by 24%, 13% and 11%, for concentrations of 25, 50 and 75 μM of Se respectively, whereas the concentration of 100 μmol L^{-1} of Se did not statistically differ from the control. Reduction of Ca, K and S levels was found in several wild plants when applying Se in the form of selenate[51].

The contents of K and S in the tubers were affected by the sources of Se, not having

the selenato presenting statistical difference in relation to the control; thus, selenite promoted a reduction of 14% for K content (Fig. 3c) and 6% the S content (Fig. 3d), when compared to selenate. Research on the application of Se through soil and hydroponic system showed an increase in S content in lettuce plants[8], rice[9] and wheat[16] when Se was applied in the form of selenate. This increase, according to some researchers, may be related to the synergism existing between these chemical elements; since selenate and sulfate have chemical similarities, the plants absorb and assimilate Se through the same metabolic route of S[36,52]. However, no research was found relating the effect of leaf application of Se with the S content in plants.

In relation to micronutrients, the application of selenate increased by 11% the B content in the aerial part of the plants when compared to selenite that did not differ of control (Fig. 3e); as well as increased by 124% the Fe content in the tubers, when compared to selenite and incresead of 67% in relation to control (Fig. 3f). Increase in Fe content was observed in rice grains[9] by applying 50 μmol L^{-1} of Se via foliar in the form of selenate and in wheat[34] by providing 40 mg L^{-1} of Se also via leaf. On the other hand, a reduction in Fe content was observed in wild plants when supplied with 50 g ha^{-1} of Se[51].

Physico-chemical characteristics of tubers

Among the physico-chemical characteristics evaluated in the potato tubers, only the soluble solids (SS) and the maturation index (MI) were influenced by the foliar application of Se (Fig. 4). The SS were affected only by the sources of Se, and selenate increased the SS by 5% in relation to control and 18% when compared to selenite (Fig.

4a). On the other hand, the MI was affected only by the concentrations of Se; the highest value of this index was detected when 100 μmol L^{-1} of Se was applied (Fig. 4b), with a 15% increase in relation to the control plants. The other doses did not statistically differ from the control plants.

Reduction in tomato fruit maturation was observed when the plants were grown in nutrient solution with 1 mg L^{-1} of Se in the form of selenite[14]. This might have been caused by the reduction in ethylene production and higher concentration of acids, because these are spent in the breath and/or in the process of maturation of the fruits, being converted into sugars[14].

High Se concentrations have a pro-oxidant effect on plants, which implies a greater maturation and senescence of the plants[36,37], as evidenced by the increase in the MI (Fig. 4b) when the highest concentration of the element is provided.

Since research relating the effect of the application of Se on physical-chemical characteristics of vegetable crops are incipient, the study of characteristics such as the content of SS and MI in fruits and tubers of biofortified plants with Se is of great importance because this element, when provided at low doses, reduces the production of ethylene[14], which is linked to fruit maturation. Nevertheless, at high doses it can accelerate the maturation process of the products, which may compromise the technological quality of the food and thereby reduce the acceptance of the biofortifieds products by consumers.

Biochemical characteristics of tubers

In plant aerobic metabolism, which occur mainly in the mitochondria, chloroplasts and

peroxisomes, O_2 inevitably leads to the formation of reactive oxygen species (ROS), such as the superoxide anionic radical ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2) and the hydroxyl radical $OH^{\bullet}$[53]. In biological systems, these ROS can cause modifications in the nitrogenous bases, promoting inactivation or DNA mutation and denaturation of proteins by the oxidation of sulfhydryl (-SH) groups or disulfide (-S-S-) bridges, removing hydrogen atoms from fatty acids components of cell membranes, and thereby initiating lipid peroxidation[54].

Several studies have shown that the antioxidant system is able to activate the activity of antioxidant enzymes, reducing ROS formation and lipid peroxidation in plant cells[8,55-57]. Plants have a defense system against ROS, composed of antioxidant enzymes such as Superoxide Dismutase (SOD), Catalase (CAT), Ascorbate Peroxidase (APX), Peroxidases (POD), Polyphenoloxidase (PPO), among others. Therefore, the adoption of practices (application of Se in plants) that favor the activation of enzymes of the antioxidant system of plants seems to be a favorable alternative to improve the development of plants in environments that may cause some stress and compromise the production.

The Fig. 5 shows the effects of the application of the sources and concentrations of Se via foliar on potato plants in the activity of enzymes and compounds linked to the vegetal antioxidant system. The SOD activity was influenced by the sources of Se; the concentration of H_2O_2 and lipid peroxidation by the interaction between sources and concentrations of Se and the activities of POD and PPO were influenced by the concentrations of Se. The activities of APX and CAT were not influenced by the treatments (data not shown).

The application of selenate did not differ of the control and the selenite aplication reduced the SOD activity in 26% when compared to selenate and control (Fig. 5a). Superoxide Dismutase (SOD) is not a selenoprotein, but the presence of Se increases the gene expression and the activity levels of that enzyme. In this sense, it was reported that the genes involved in the mechanisms of activation of SOD were significantly regulated in maize roots 24 hours after the application of 1 μmol L^{-1} of Se[58].

The increase in SOD activity after the application of Se represents an evidence that the element is involved in the elimination of superoxide radicals and hydroxyl radicals in cells[34], since this enzyme is considered the first line of defense against the ROS promoted by the dismutation of $O_2^{\cdot -}$ in $H_2O_2 + O_2$. The produced H_2O_2 is converted in $H_2O + O_2$ by a series of peroxidases such as CAT, APX, POD and PPO, thereby neutralizing the deleterious effects of ROS on plants[54].

The application of lower concentrations of Se in the form of selenate promoted reduction of 29.5%; 29.8% and 33.5% in the H_2O_2 in relation to the control, for the concentrations of 25, 50, 75 μmol L^{-1}, respectively; and these were not statistically different from each other (Fig. 5b). However, the application of 100 μmol L^{-1} of Se in the selenate form promoted an increase of H_2O_2 levels in tubers in relation to the lower concentrations of Se applied, being, however, inferior to the one found in the control plants. For the application of selenite, the H_2O_2 concentration did not statistically differ from the control plants. However, this source promoted higher values of H_2O_2 when compared to selenate for all applied concentrations.

In plants of lettuce[8] and brachiaria[57] submitted to low concentrations of Se in the form of selenate, an increase in SOD and CAT activity was observed, with a reduction in

the activity of these enzymes when the concentrations of Se were increased. In soybean plants[55] was observed increased productivity and reduced chlorophyll degradation after foliar application of sodium selenate (50 mg L^{-1}) at 78 days after emergence.

The POD and PPO activities were influenced by the concentrations of Se regardless the source (Fig. 5c, d). The application of Se increased the activity of POD reaching at the highest applied concentration (100 μmol L^{-1} Se) a value 25% higher than the control (Fig. 5c). These results corroborate those of researches with other plant species, in which the increase in the concentration of Se resulted in greater enzymatic activity of POD[13]. The application of 5 μmol L^{-1} of Se in the form of selenite increased the activity of POD in ryegrass plants cultivated in hydroponics, whereas in the lower concentrations it was observed a lower activity of this enzyme[56].

The POD is directly related to the senescence of plants and the more mature or older parts present higher activity of this enzyme[59]. Moreover, high doses Se present pro-oxidant effect[15], increasing the maturation and senescence of the fruits. This was confirmed in this study with potato, in which the tubers presented higher MI (Fig. 4b) and higher POD activity (Fig. 5c) when submitted to the highest concentration of Se (100 μmol L^{-1}).

For the PPO activity (Fig. 5d) only the highest concentration of Se (100 μmol L^{-1}) statistically differed from the control plants, with a 44% reduction. The PPO activity is influenced by several factors, among them the maturity of the plants, presenting higher values in younger plant tissues[60]. This fact was verified in the present study, in which the application of 100 μmol L^{-1} of Se resulted in greater maturation of the tubers (Fig. 4b) and in lower PPO activity (Fig. 5d).

Lipid peroxidation was not affected by the application of selenite, regardless of the used concentration. On the other hand, the application of selenate in the lowest concentrations resulted in a reduction of 30%, 24% and 22% in MDA concentration when 25, 50 and 75 μmol L^{-1} of Se were applied respectively, while the highest concentration of Se (100 μmol L^{-1}) did not statistically differ from the control (Fig. 5e). In bean plants seedlings in hydroponics was observed that the concentrations of 4 mg L^{-1} and 6 mg L^{-1} increased the H_2O_2 and the MDA concentration[61]. High Se contents can cause the increase of ROS concentration in the plant cells[15,36,37,57], among them H_2O_2, which is considered one of the most harmful ROS to plant metabolism, as it causes damage to cell membranes and, therefore, compromises physiological and metabolic activities.

In spite of the positive results in relation to Se foliar application for potato biofortification, future work under field conditions should be carried out in order to establish an ideal concentration of Se to be applied in potato plants, especially because experiments with vegetable crops are still scarce. It is also necessary the development of researches to evaluate the anatomy of the plants and to identify the damages caused to the plant cells by the high concentrations of Se, especially with the elevation of the concentration of H_2O_2 and lipid peroxidation. It is still necessary to carry out sensorial tests to identify changes in color, cooking time and flavor of tubers, and more importantly, to identify the acceptance rate of biofortified foods by the consumers.

Conclusions

Selenate elevates the internal carbon concentration and the stomatal conductance in

potato plants, provides higher levels of B in the aerial part of the plants, increases the levels of K, S, Fe, SS and SOD activity and decrease H_2O_2 and lipid peroxidation in the tubers.

The application of selenite increases the content and accumulation of Se in the shoot and increases the concentration of H_2O_2 and MDA in the tubers.

The application of different concentrations of Se, regardless of the source, increases its content in the tubers, causes an increase in the photosynthetic rate, maturation index and POD activity in tubers and reduces the Mg content in shoot and the PPO activity in potato tubers.

References

1. Rayman, M. P. Selenium and human health. *Lancet.* **379,** 1256-1268 (2012).

2. Kipp, A. P. *et al*. Revised reference values for selenium intake. *J. Trace Elem. Med. Biol.* **32,** 195-199 (2015).

3. Loscalzo, J. Keshan disease, selenium deficiency, and the selenoproteome. *N. Engl. J. Med.* **370,** 1756-1760, (2014).

4. Zhang, G. *et al*. Genetic Associations with Gestational Duration and Spontaneous Preterm Birth. *N. Engl. J. Med.* **377,** 1156-1167 (2017).

5. Marcocci, C. *et al*. Selenium and the Course of Mild Graves' Orbitopathy. *N. Engl. J. Med.* **364,** 1920-1931, (2011).

6. Almondes, K. G. S. *et al*. O papel das selenoproteínas no câncer. *Rev. Assoc. Med. Bras.* **56,** 484-488 (2010).

7. Kápolna, E. *et al*. Effect of foliar application of selenium on its uptake and speciation

in carrot. *Food Chem.* **115,** 1357-1363 (2009).

8. Ramos, S. J. *et al*. Selenium biofortification and antioxidant activity in lettuce plants fed with selenate and selenite. *Plant Soil Environ.* **56,** 584-588 (2010).
9. Boldrin, P. F. *et al*. Soil and foliar application of selenium in rice biofortification. *J. Food Compost. Anal.* **31,** 238-244 (2013).
10. Deng, X. *et al*. Difference of selenium uptake and distribution in the plant and selenium form in the grains of rice with foliar spray of selenite or selenate at different stages. *Field Crops Res.* **211,** 165-171 (2017).
11. Mäkelä, A. L. *et al*. Environmental effects of nationwide selenium fertilization in Finland. *Biol. Trace Elem. Res.* **47,** 289-298 (1995).
12. Li, H. F., McGrath, S. P. & Zhao F. J. Selenium uptake, translocation and speciation in wheat supplied with selenate or selenite. *New Phytol.* **178,** 92-102 (2008).
13. Mora, M. L. L., Pinilla, L., Rosas, A. & Cartes P. Selenium uptake and its influence on the antioxidative system of white clover as affected by lime and phosphorus fertilization. *Plant Soil* **303,** 139-149 (2008).
14. Zhu, Z. *et al*. Selenium delays tomato fruit ripening by inhibiting ethylene biosynthesis and enhancing the antioxidant defense system. *Food Chem.* **219,** 179-184 (2017).
15. Hartikainen, H., Xue, T. & Piironen, V. Selenium as an anti-oxidant and pro-oxidant in ryegrass. *Plant Soil* **225,** 193-200 (2000).
16. Boldrin, P. F. *et al*. Selenium promoters sulfur accumulation and plant growth in wheat (*Triticum aestivum*). *Physiol. Plantarum* **158,** 80-91 (2016).
17. Food and Agriculture Organization of the United Nations, FAO-FAOSTAT

http://faostat.fao.org/faostat (2013).

18. Turakainen, M., Hartikainen, H. & Seppänen, M. M. Effects of selenium treatments on potato (*Solanum tuberosum* L.) growth and concentrations of soluble sugars and starch. *J. Agric. Food Chem.* **52,** 5378-5382 (2004).
19. Ježek, P. *et al*. Effect of foliar application of selenium on the content of selected amino acids in potato tubers (*Solanum tuberosum* L.). *Plant Soil Environ.* **57,** 315-320 (2011).
20. U.S. Environmental Protection Agency, USEPA. Method 3051A – Microwave assisted acid digestion of sediments, sludges, soils, and oils. https://www.epa.gov/sites/production/files/2015-12/documents/3051a.pdf (2007).
21. Malavolta, E., Vitti, G. C., Oliveira, S. A. *Avaliação do Estado Nutricional das Plantas: Princípios e Aplicações*. (Potafos, 1997).
22. Association of Official Analytical Chemists, AOAC. Official Methods of Analysis of the Association of the Agricultural Chemists. 18th ed. (AOAC International, 2010).
23. Braun, H., Fontes, P. C. R., Finger, F. L., Busato, C. & Cecon, P. R. Carboidratos e matéria seca de tubérculos de cultivares de batata influenciados por doses de nitrogênio. *Ciênc. Agrotec.* **34,** 285-293 (2010).
24. Matsumo, H. & Uritani, I. Physiological behavior of peroxidase enzymes in sweet potato root tissue injured by cutting or black root. *Plant. Cell. Physiol.* **13,** 1091-1101 (1972).
25. Teisson, C. Lê brunissement interne de I'ananás. I-Historique. II-Material et methodos. *Fruits* **34,** 245-281 (1979).
26. Biemelt, S., Keetman, U. & Albreacht, G. Re-aeration following hypoxia or anoxia

leads to activation of the antioxidative defense system in roots of wheat seedlings. *Plant Physiol.* **116,** 651-658 (1998).

27. Giannopolitis, C. N. & Ries, S. K. Superoxide dismutases: I. Occurrence in higher plants. *Plant Physiol*. **59,** 309-314 (1977).
28. Havir, E. A. & McHale, N. A. Biochemical and developmental characterization of multiple forms of catalase in tobacco leaves. *Plant Physiol.* **84,** 450-455 (1987).
29. Nakano, Y. & Asada, K. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant. Cell. Physiol*. **22,** 867-880 (1981).
30. Velikova, V., Yordanov, I. & Edreva, A. Oxidative stress and some antioxidant systems in acid rain-treated bean plants protective role of exogenous polyamines. *Plant Sci.* **151,** 59-66 (2000).
31. Buege, J. A. & Aust, S. D. Microsomal lipid peroxidation. *Methods in Enzymol.* **52,** 302-310 (1978).
32. Scott, A. J. & Knott, M. A cluster analysis method for grouping means in the analysis of variance. *Biometrics* **30,** 507-512 (1974).
33. R Core Team. R: A language and environment for statistical computing R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org (2015).
34. Nawaz, F., Ahmad, R., Ashraf, M. Y., Waraich, E. A. & Khan, S. Z. Effect of selenium foliar spray on physiological and biochemical processes and chemical constituents of wheat under drought stress. *Ecotox. Environ. Safe.* **113,** 191-200 (2015).
35. Alyemeni, M. N., Ahanger, M. A., Wijaya, L., Alam, P., Bhardwaj, R. & Ahmad, P. Selenium mitigates cadmium-induced oxidative stress in tomato (*Solanum*

lycopersicum L.) plants by modulating chlorophyll fluorescence, osmolyte accumulation, and antioxidant system. *Protoplasma* **255,** 459-469 (2018).

36. Pilon-Smits, E. A. H., Quinn, C. F., Tapken, W., Malagoli, M. & Shiavon, M. Physiological functions of beneficial elements. *Curr. Opin. Plant Biol.* **12,** 267-274 (2009).
37. Schiavon, M., Lima, L. W., Jiang, Y. & Hawkesford, M. J. Effects of Selenium on Plant Metabolism and Implications for Crops and Consumers. In: Pilon-Smits, E. A. H. *et al*. (eds.). *Selenium in Plants*. 257-275 (Springer, 2017).
38. Beaulieu, J. M., Leitch, I. J., Patel, S., Pendharkar, A. & Knight, C. A. Genome size is a strong predictor of cell size and stomatal density in angiosperms. *New Phytol.* **179,** 975-986 (2008).
39. Franks, P. J., Drake, P. L. & Beerling, D. J. Plasticity in maximum stomatal conductance constrained by negative correlation between stomatal size and density: An analysis using *Eucalyptus globulus*. *Plant Cell Environ*. **32,** 1737-1748 (2009).
40. Drake, P. L., Froend, R. H. & Franks, P. J. Smaller, faster stomata: Scaling of stomatal size, rate of response, and stomatal conductance. *J. Exp. Bot*. **64,** 495-505 (2013).
41. Raven, J. A. Speedy small stomata? *J. Exp. Bot*. **65,** 1415-1424 (2014).
42. Lyons, G., Ortiz-Monasterio, I., Stangoulis, J. & Graham, R. Selenium concentration in wheat grain: is there sufficient genotypic variation to use in breeding? *Plant Soil* **269,** 369-380 (2005).
43. Ahmad, R., Waraich, E. A, Nawaz, F., Ashraf, M. Y. & Khalid, M. Selenium (Se) improves drought tolerance in crop plants - a myth or fact? *J. Sci. Food Agric.* **96,**

372-380 (2016).

44. Seppänen, M. M. *et al*. Agronomic biofortification of *Brassica* with selenium-enrichment of SeMet and its identification in *Brassica* seeds and meal. *Plant Soil* **337,** 273-283 (2010).

45. Cartes, P., Gianfreda, L. & Mora, M. L. Uptake of selenium and its antioxidant activity in ryegrass when applied as selenate and selenite forms. *Plant Soil* 276, 359-367 (2005).

46. Shalaby, T. *et al*. Selenium fortification induces growth, antioxidant activity, yield and nutritional quality of lettuce in salt-affected soil using foliar and soil applications. *Plant Soil* **421,** 245-258 (2017).

47. Zayed, A., Lytle, C. M. & Terry, N. Accumulation and volatilization of different chemical species of selenium by plants. *Planta* **206,** 284-292 (1998).

48. Poggi, V., Arcioni, A., Filippini, P. & Pifferi, P. G. Foliar application of selenite and selenate to potato (*Solanum tuberosum*): effect of a ligand agent on selenium content of tubers. *J. Agric. Food Chem.* **48,** 4749-4751 (2000).

49. Kápolna, E., Hillestrøm, P. R., Laursen, K. H., Husted, S. & Larsen, E. H. Effect of foliar application of selenium on its uptake and speciation in carrot. *Food Chem.* **115,** 1357-1363 (2009).

50. U.S. Department of Agriculture, Agricultural Research Service. USDA National Nutrient Database for Standard Reference, Release 25. Nutrient Data Laboratory Online. https://www.ars.usda.gov/northeast-area/beltsville-md-bhnrc/beltsville-human-nutrition-research-center/nutrient-data-laboratory/docs/sr25-home-page/ (2012).

51. Drahonovský, J. *et al*. Selenium uptake, transformation and inter-element interactions by selected wildlife plant species after foliar selenate application. *Environ. Exp. Bot.* **125,** 12-19 (2016).

52. Sors, T. G., Ellis, D. R. & Salt, D. E. Selenium uptake, translocation, assimilation and metabolic fate in plants. *Photosynth. Res.* **86,** 373-389 (2005).

53. Marschner, H. *Marschner's Mineral Nutrition of Higher Plants* (ed. Marschner, P.) (Academic Press, 2012).

54. Barbosa, M. R., Silva, M. M. de A., Willadino, L., Ulisses, C. & Camara, T. R. Geração e desintoxicação enzimática de espécies reativas de oxigênio em plantas. *Ciência Rural* **44,** 453-460 (2014).

55. Djanaguiraman, M., Devi, D. D., Shanker, A. K., Sheeba, J. A. & Bangarusamy, U. Selenium: an antioxidative protectant in soybean during senescence. *Plant Soil* **272,** 77-86 (2005).

56. Cartes, P., Jara, A. A., Pinilla, L., Rosas, A. & Mora, M. L. Selenium improves the antioxidant ability against aluminium-induced oxidative stress in ryegrass roots. *Ann. Appl. Biol.* **156,** 297-307 (2010).

57. Ramos, S. J. *et al*. Response of brachiaria grass to selenium forms applied in a tropical soil. *Plant Soil Environ.* **58,** 521-527 (2012).

58. Jiang, C. *et al*. Effect of exogenous selenium supply on photosynthesis, Na^+ accumulation and antioxidative capacity of maize (*Zea mays* L.) under salinity stress. *Sci. Rep.* **7,** 42039; 10.1038/srep42039 (2017).

59. Singh, R. & Dwivedi, U. N. Effect of ethrel and 1methylcyclopropene (1MCP) on antioxidants in mango (*Mangifera indica* var. Dashehari) during fruit ripening. *Food*

Chem. **111,** 951-956 (2008).

60. Nicolas, J. J., Richard-Forget, F. C., Goupy, P. M., Amiot, M. J. & Aubert, S. Y. Enzymatic browning reactions in apple and apple products. Crit. Rev. *Food Sci. Nutr.* **34,** 109-157 (1994).
61. Aggarwal, M. *et al*. Exogenous proline application reduces phytotoxic effects of selenium by minimising oxidative stress and improves growth in bean (*Phaseolus vulgaris* L.) seedlings. *Biol. Trace Elem. Res.* **140,** 354-367 (2011).

Acknowledgment

National Council for Technological Development (CNPq-Brazil), the Coordination of Improvement of Higher Level Personnel (CAPES) and the Foundation for Research Support of Minas Gerais (FAPEMIG) for financial support.

Author Contributions

Oliveira Vanuze and Faquin Valdemar designed the study. Oliveira Vanuze and Faquin Valdemar analysed and interpreted the data. Oliveira Vanuze and Teodoro Jéssica conducted the greenhouse and laboratory works. Oliveira Vanuze and Faquin Valdemar participated in the sample collection. Oliveira Vanuze, Faquin Valdemar, Pereira Joelma, Guilherme Luiz and Teodoro Jéssica wrote the manuscript, and Guilherme Luiz revised the manuscript. All the authors read and approved this paper.

Additional Information

Competing Interests: The authors declare no competing interests.

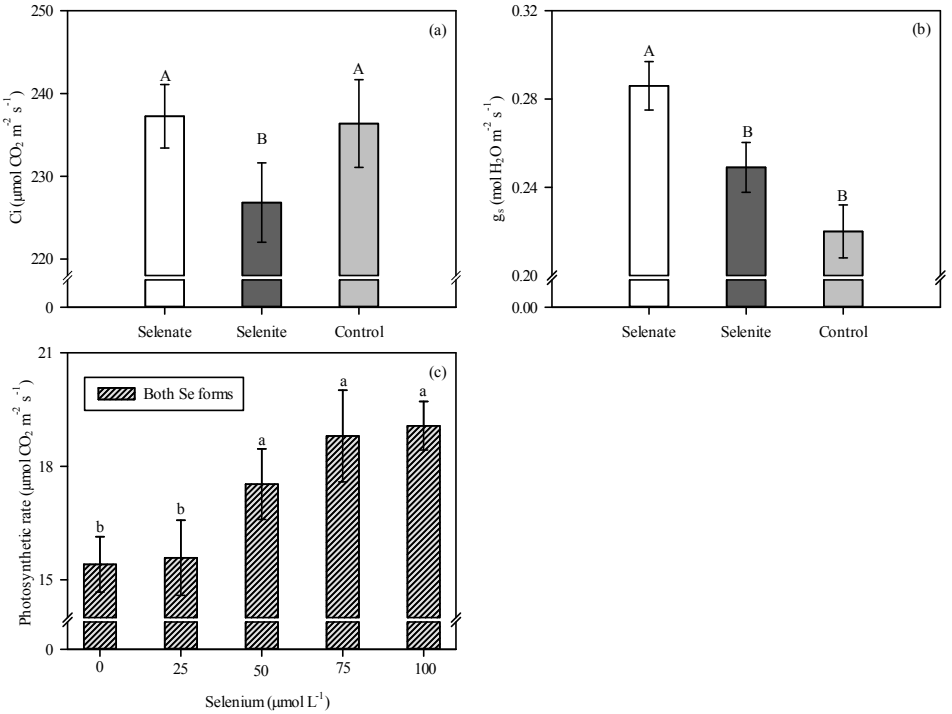

Fig. 1 Effect of Se applied via foliar in the internal concentration of CO_2 - *Ci* (a), stomatal conductance - *gs* (b) and photosynthetic rate - *A* (c) of potato plants. Same letters, upper case comparing sources and lower case comparing Se concentrations, do not differ by the Scott-Knott's test ($p \leq 0.05$). Vertical bar indicates the standard error of the mean (n = 4).

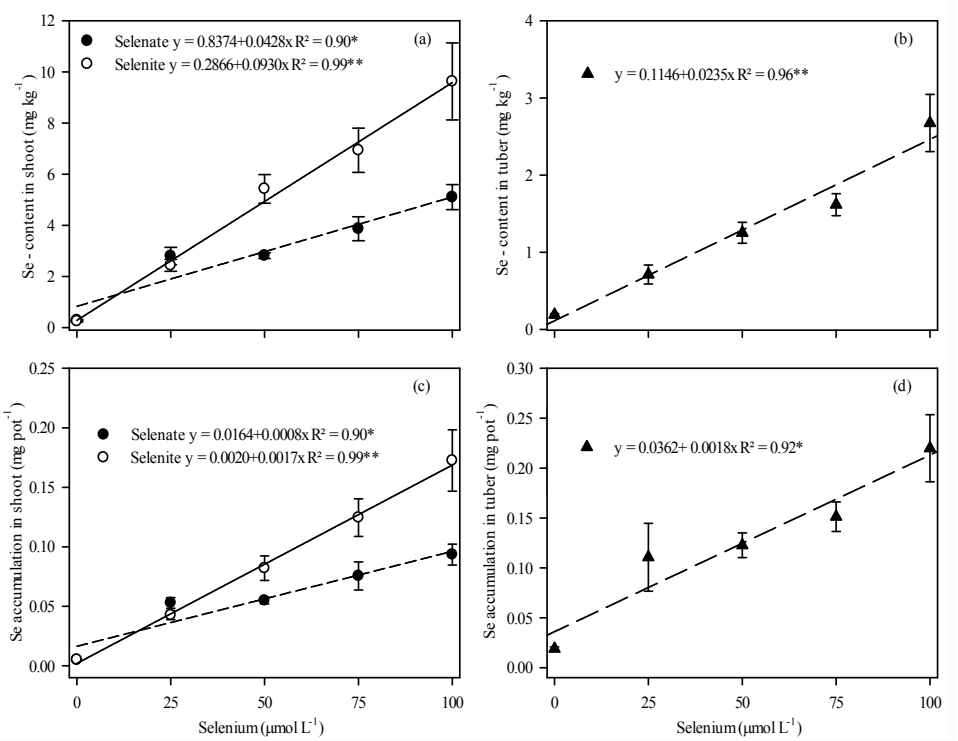

Fig. 2 The content of Se in shoot and tubers (a, b) and accumulation of Se in shoot and tubers (c, d) of potato plants as a function of concentrations and sources of Se applied via foliar. Vertical bar indicates the standard error of the mean (n = 4).

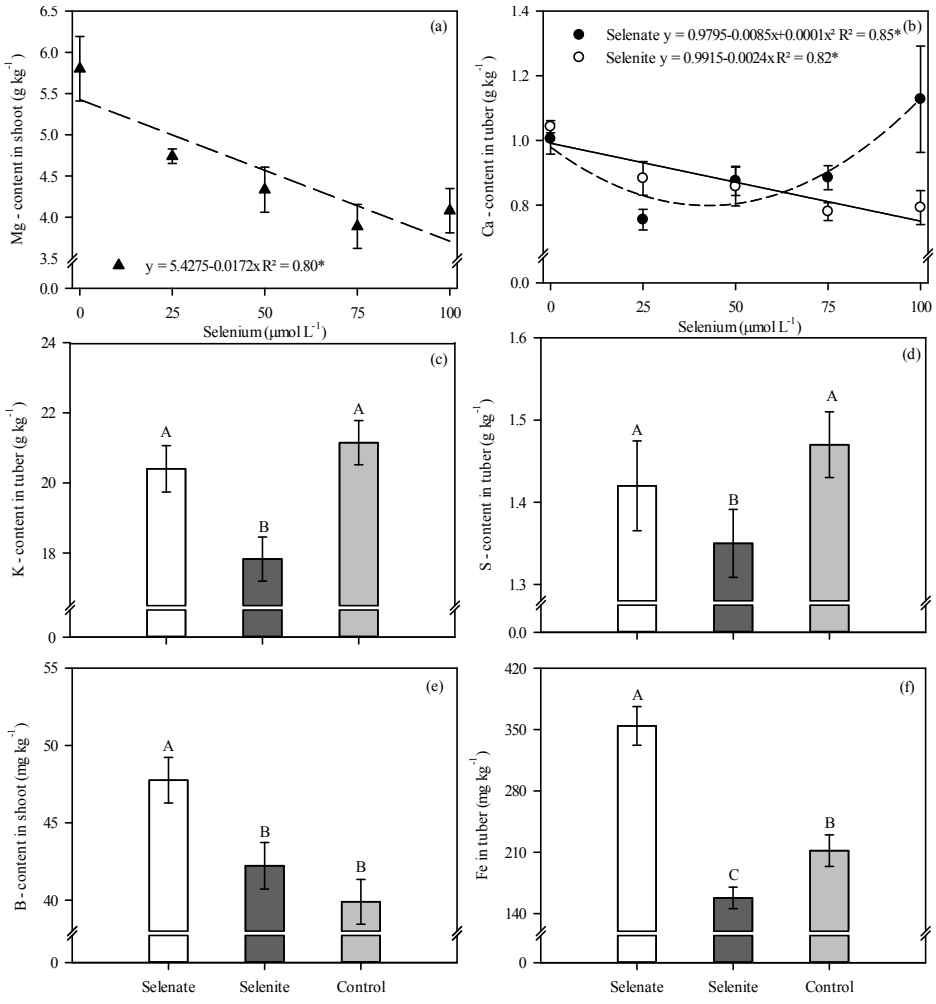

Fig. 3 Magnesium - Mg content in shoot of the plants (a) and calcium - Ca in tubers (b) as a function of Se concentrations; and potassium contents - K (c), sulfur - S (d), boron - B (e) and iron - Fe (f) in potato tubers as a function of seed source. Same letters, upper case comparing sources and lower case comparing Se concentrations, do not differ by the Scott-Knott's test ($p \leq 0.05$). Vertical bar indicates the standard error of the mean (n = 4).

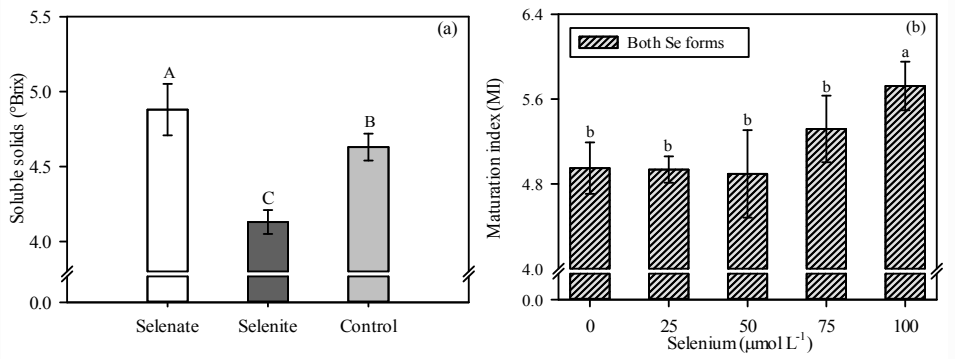

Fig. 4 Effect of foliar application of Se on soluble solids content (a) and maturation index (b) of potato tubers. Same letters, upper case comparing sources and lower case comparing Se concentrations, do not differ by the Scott-Knott's test ($p \leq 0.05$). Vertical bar indicates the standard error of the mean (n = 4).

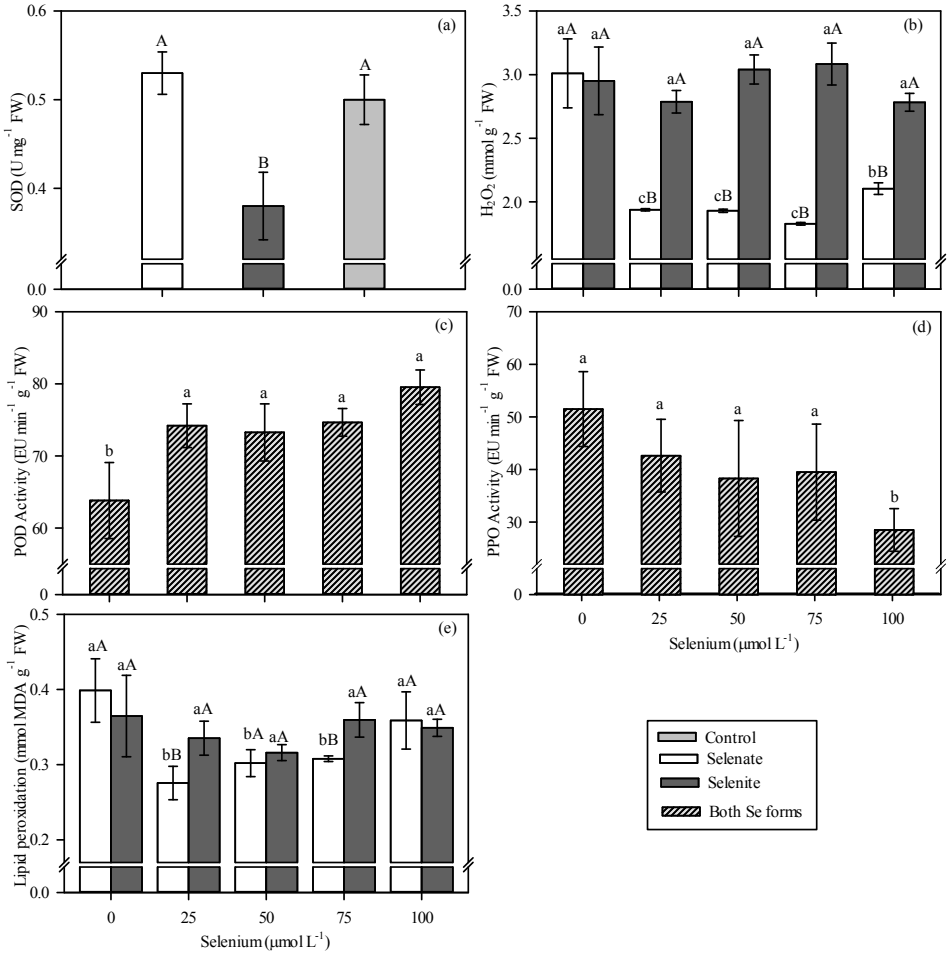

Fig. 5 Effect of foliar application of Se in the enzymatic activity of SOD (a), hydrogen peroxide (b), peroxidase (c), polyphenoloxidase (d) and lipid peroxidation (e) in potato tubers. Same letters, upper case comparing sources and lower case comparing Se concentrations, do not differ by the Scott-Knott's test ($p \leq 0.05$). Vertical bar indicates the standard error of the mean (n = 4).