UNIVERSIDADE FEDERAL DE LAVRAS

MÁRCIO FELIPE PINHEIRO NERI NUNES

STRATEGIES FOR AGRONOMIC BIOFORTIFICATION WITH SELENIUM AND IODINE IN SWEET POTATO GROWN IN TROPICAL SOIL

LAVRAS – MG
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Thesis presented to the Federal University of Lavras, as part of the requirements of the Graduate Program in Soil Science area of concentration in Soil Fertility and Plant Nutrition, to obtain the title of Doctor.

Professor D.Sc. Guilherme Lopes
Advisor
Professor Ph.D. Matthew G. Siebecker
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ESTRATÉGIAS PARA BIOFORTIFICAÇÃO AGRONÔMICA COM SELÊNIO E IODO EM BATATA DOCE CULTIVADA EM SOLO TROPICAL

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APROVED in September, 19th, 2023

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2023

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

Nunes, Márcio Felipe Pinheiro Neri.
Strategies for agronomic biofortification with selenium and iodine in sweet potato grown in tropical soil / Márcio Felipe Pinheiro Neri Nunes. - 2023.
80 p. : il.

Orientador(a): Guilherme Lopes.
Coorientador(a): Matthew G. Siebecker.
Tese (doutorado) - Universidade Federal de Lavras, 2023.
Bibliografia.

1. Ipomoea batatas L. 2. Selenate. 3. Beneficial element. I. Lopes, Guilherme. II. Siebecker, Matthew G. III. Título.

A Deus por estar presente em todos os momentos da minha vida.

Às minhas duas avós Maria Neri (In memoriam) e Marinita Pinheiro (In memoriam), minhas primeiras professoras.

Ao meu avô Vicente Gracindo (In memoriam), pelo incentivo através do cultivo da terra.

Aos meus pais Jeovânia Pinheiro e Marcio Neri, pelo exemplo de vida e incentivo ao estudo desde a infância.

Aos meus irmãos Luiz Gustavo e Carmem Lúcia, pelo amor fraternal e companheirismo.

A todos os professores e orientadores que me acompanharam até este momento.

A toda minha família e amigos.

DEDICO

AGRADECIMENTOS

Agradeço primeiramente a Deus. A minha família, que sempre foi meu esteio em todos os momentos, a minhas avós Maria Neri Leite Nunes (*In memoriam*) e Marinita Pinheiro Gracindo (*In memoriam*), a meu avô Vicente Gracindo Filho (*In memoriam*), a minha mãe Maria Jeovânia Pinheiro Gracindo, a meu pai Marcio Neri Nunes, ao meu irmão Luiz Gustavo Pinheiro Neri Nunes, a minha irmã Carmem Lúcia Pinheiro Neri Nunes por serem meus maiores incentivadores e minha maior motivação em todos os momentos desta caminhada.

A todos os familiares não citados diretamente, peço que se considerem como tal, pois são parte integrante de todo este processo.

A Universidade Federal de Lavras (UFLA), especialmente ao Departamento de Ciência do Solo (DCS), pela oportunidade da formação como Doutor em Ciência do Solo.

Ao CNPq, Conselho Nacional de Desenvolvimento Científico e Tecnológico CAPES, pela concessão da bolsa de doutorado – Processo (141057/2019-0), a (CAPES) Coordenação de Aperfeiçoamento de Pessoal de Nível Superior e a Fundação de Amparo à Pesquisa de Minas Gerais (FAPEMIG), pelos recursos destinados a execução deste projeto.

Ao meu orientador Prof. DSc. Guilherme Lopes por toda a confiança em mim depositada, compreensão, conhecimento, estímulo, bem como todos os valores profissionais e pessoais que me foram ensinados durante todo esse processo.

A todos os professores do DCS/UFLA que contribuíram para a esta formação.

Ao professor PhD Matthew G. Siebecker da Texas Tech University (TTU), e todos os pesquisadores do Soil Chemistry Laboratory - Department of Plant and Soil Science da TTU por todos ensinamentos, conselhos e contribuições durante o doutorado sanduíche nos EUA.

A todos os técnicos e funcionários do DCS/UFLA, ao Laboratório de Química Ambiental e o Laboratório de Nutrição Mineral de Plantas da UFLA, em especial, aos técnicos (as) Alexandre, Aline, Bethânia, Geila, Lívia e Mariene por toda paciência e dedicação no acompanhamento e realização das análises desta tese.

A todos os alunos do DCS/UFLA, de modo especial aos integrantes do grupo de pesquisa, Cynthia, Gabrielly, Guilherme, Josimar, Leônidas, Mateus, Raul e Ruby, e aos alunos de iniciação científica Danilo, Fernanda, Gabryel e Iuri, pelo saudável e harmonioso convívio no ambiente de trabalho e estudo.

À Fazenda 3W Agronegócios e seus funcionários pela implementação e condução do experimento de tese.

À empresa SQM em nome de pesquisadora Katja Hora e à Faculty of Science, University of Nottingham em nome da pesquisadora Liz Bailey por toda a colaboração e suporte nas análises do experimento.

Ao professor DSc. Valter Carvalho de Andrade Júnior do Departamento de Agricultura (DAG) da UFLA, e aos seus orientados Jefferson e Orlando por todo o conhecimento compartilhado sobre a cultura da batata doce.

A todas que compuseram o apartamento 2425 em Lubbock, Texas, EUA, aos colegas de república em Lavras, MG, a os amigos que compõe o Núcleo de Estudos em Ciência do Solo (NECS), pelo apoio, aprendizado e amizade, serei eternamente parte desta família.

E a todos que de alguma forma deram sua contribuição para materialização deste sonho de longa data, não poderia me sentir de outra forma se não eternamente grato.

RESUMO GERAL

A batata-doce (*Ipomoea batatas* L.) é uma cultura agrícola importante para a segurança alimentar em muitos países em desenvolvimento. Considerando que os solos tropicais são pobres em elementos essenciais ao homem, como o selênio (Se), e que concentração de um elemento na planta está diretamente relacionada à sua disponibilidade no solo, a biofortificação agronômica é uma das principais alternativas para aumentar a disponibilidade do teor de nutrientes em alimentos básicos. Neste contexto, este estudo avaliou diferentes estratégias de biofortificação agronômica de batata-doce com Se e iodo (I), visando: i) avaliar o potencial de biofortificação com Se na presença ou ausência da adição de I, em resposta ao aumento das doses de Se aplicadas via pulverização foliar; ii) avaliar a capacidade de adsorção de Se(VI) em solo tropical e sua posterior dessorção por fósforo e nitrogênio; e iii) avaliar a biofortificação de Se aplicando doses crescentes de Se no solo utilizando fertilizantes enriquecidos com Se (MAP e/ou ureia). Para atingir esses objetivos, foram realizados dois experimentos de campo utilizando as seguintes doses de Se (0, 50, 100 e 200 g ha^{-1}). O primeiro avaliou os efeitos de doses de aplicação foliar de Se(VI) na presença ou ausência de I (500 g ha^{-1}), também via pulverização foliar, em um arranjo fatorial 4x2+1, havendo um tratamento adicional com as maiores doses de Se+I e 2,5 kg ha^{-1} de zinco (Zn). O segundo avaliou as mesmas doses de Se testadas na adubação foliar, mas com aplicação via solo, usando-se fertilizantes enriquecidos com Se (Se-fosfato monoamônico) - (Se-MAP), Se-ureia, e Se-MAP+ureia. Além disso, foi testada a adsorção de Se no solo coletado na área experimental e, posteriormente sua dessorção pela adição de doses de P_2O_5 e N (equivalentes a 0, 100, 250, 500 e 1000 kg ha^{-1}) em solução, preparadas tanto com sais puros ou MAP /ureia. Os resultados demonstraram que, em ambos os estudos, os teores de Se na parte aérea e raiz da batata-doce aumentaram com o aumento das doses de Se aplicadas, sendo esses teores maiores quando o Se foi disponibilizado via foliar. A adição foliar de I não afetou o teor de Se acumulado na batata-doce, porém, o teor de I encontrado na planta foi maior quando o Se foi adicionado na dose de 50 g ha^{-1}. A aplicação simultânea de Se, I e Zn (via pulverização foliar) resultou nos maiores teores de Se na batata-doce. A partir do segundo estudo, descobriu-se que a adsorção de Se aumentou com o aumento da concentração de Se adicionado na solução e a dessorção de Se aumentou com o aumento da adição de P (como reagente p.a.) e N (ureia) em solução. As plantas de batata-doce responderam positivamente às aplicações de Se no solo, porém o maior potencial de biofortificação foi alcançado com a ureia enriquecida com Se, concordando com o teste de dessorção. A biofortificação agronômica da batata-doce com Se é promissora em solos tropicais, porém as doses de Se a serem aplicadas diferem dependendo da estratégia utilizada (foliar ou via solo).

Palavras-chave: Selenato. Iodato. Agricultura funcional. Solo tropical. Elementos benéficos.

GENERAL ABSTRACT

Sweet potato (*Ipomoea batatas* L.) is an important crop for assuring food security in many developing countries. Considering that tropical soils are poor in many human-essential elements, such as selenium (Se), and that the concentration of an element in the plant is directly related to its availability in the soil, agronomic biofortification is one of the main alternatives to increase nutrient availability in staple foods. In this context, this study evaluated different strategies for agronomic biofortification of sweet potatoes with Se and iodine (I), aiming to: i) assess the biofortification potential of Se in the presence or absence of I addition, in response to increasing Se rates applied as a foliar spray; ii) evaluate Se(VI) adsorption capacity in a tropical soil and its further desorption by phosphorus and nitrogen; and iii) assess biofortification of Se by applying increasing Se rates in the soil using Se-enriched fertilizers (MAP and/or urea). To achieve these goals, two field trials were conducted using the following Se rates (0, 50, 100, and 200 g ha^{-1}). The first experiment evaluated the effects of increasing Se(VI) foliar application rates in the presence or absence of I (500 g ha^{-1}), also applied as foliar spray), as a factorial design 4x2+1, with an additional treatment consisting of the higher rates of Se+I and 2.5 kg ha^{-1} of zinc (Zn). The second assessed applications of the same Se rates (*i.e.,* 0, 50, 100, and 200 g ha^{-1}), but the application was carried out in the soil using Se-enriched fertilizers (Se-monoammonium phosphate) - (Se-MAP), Se-urea, and Se-MAP+urea. Also, a Se adsorption test was conducted on soil samples collected from the field experiment, with Se desorption being evaluated by adding increasing rates of P_2O_5 and N (equivalents to 0, 100, 250, 500, and 1000 kg ha^{-1}) using both, desorption solutions prepared with pure salts or MAP/urea. Results demonstrated that, in both studies, Se contents in sweet potato shoots and roots increased upon increasing Se rates, with these contents being higher when Se was added as a foliar spray. The foliar addition of I did not affect Se contents accumulated in sweet potato, yet the I content found in the plant was higher when Se was added at 50 g ha^{-1}. The simultaneous application of Se, I, and Zn (as a foliar spray) resulted in the highest Se contents determined in sweet potatoes. The second study showed that Se adsorption increased with increasing Se concentrations added in solution, while Se desorption was greater following the addition of P (as a pure reagent) and N (urea) in solution. Sweet potato plants responded positively to applications of Se in the soil, but the highest biofortification potential was achieved with Se-enriched urea, agreeing with the desorption test. Agronomic biofortification of sweet potatoes with Se is promising in tropical soils, however, Se rates to be applied should be different depending on the strategy used (foliar or via soil).

Keywords: Selenate. Iodate. Functional agriculture. Tropical soil. Beneficial elements.

SUMÁRIO

FIRST PART

1 GENERAL INTRODUCTION

Deficiencies of selenium (Se), iodine (I), and zinc (Zn) represent a serious global health problem because these micronutrient deficiencies are affecting more than two billion of the global population, especially in developing countries (BAILEY et al., 2015; BOUIS et al., 2017; HARDING et al., 2018). The main cause of low micronutrient intake in humans occurs usually by the consumption of products grown in regions where soils are naturally poor in micronutrients (WELCH et al., 2013).

Zinc deficiency has been suggested as the main cause of the death of hundreds of thousands of children under five years of age, mainly due to enhanced infectious diseases such as diarrhea and pneumonia (BLACK et al., 2013). Zinc has strong antiviral and antibacterial effects on the human body and its presence improves the immune system by combating various viruses, including the virus responsible for COVID-19 (READ et al., 2019; SKALNY et al., 2020).

Iodine malnutrition is responsible for thyroid disorders in human populations and is often associated with a lack of I in table salt (PEARCE et al., 2016; KÖHRLE, 2023). Other disorders generated by low consumption of this element can affect important cognitive, neural, cardiac, and reproductive functions (ZIMMERMANN et al., 2008). Because of this, in 1993 the United Nations and the World Health Organization recommended the iodization of table salt, although this practice has been adopted in some countries in recent decades (UNICEF, 1994; CHARLTON et al., 2013; FUGE; JOHNSON, 2015).

The addition of I to table salt has been successfully done in most countries where it has been implemented due to its cost-benefit, however, the processing of foods with iodized salt is questioned due to its instability, caused by losses of this element through volatilization during storage, transportation, and cooking of food (SURI et al., 2016; GARCIA-CASUAL et al., 2017). Furthermore, there is a broad awareness campaign to reduce salt consumption in food as a way of preventing hypertension and cardiovascular diseases (ZIMMERMANN et al., 2012). It is noted that despite all efforts, I deficiency continues to be a public health problem and its supplementation is still required in some populations (ANDERSSON et al., 2012; GONZALI et al., 2017).

Similarly, Se is also required by mammals and acts by replacing sulfur (S) in amino acid structures, forming selenoproteins (BAÑUELOS, et al., 2022). These proteins act as antioxidants, reducing the harmful effects of free radicals (ASHRAF et al., 2018). They also support the proper functioning of the thyroid gland, preventing various types of cancer, and strengthening the immune and reproductive systems (RAYMAN, 2012).

Although some elements are not considered essential for plants, only for humans and animals, they are beneficial and can have positive effects on plant development and growth, such as Se and I. Generally, the application of these elements in low concentrations to plants helps in their development, metabolism, and flowering, as well as other benefits (WANG et al., 2013; DAI et al., 2020; GONNELLA et al., 2019; SARROU et al., 2019).

Nowadays, increasing the nutritional value of foods and, as a result, combating malnutrition, is a major challenge and agricultural approaches can be adopted to mitigate this problem, such as plant breeding and/or agronomic strategies. Agronomic biofortification consists of adding a missing element, via soil or foliar, with the aim of increasing its content in the edible parts of the crop of interest. This technique of modern agriculture represents a useful, low-cost, and sustainable strategy to combat micronutrient deficiencies in human populations. Generally, elements, such as Se, are accumulated in plant tissue as organic forms and these forms are more stable than inorganic ones available in supplementation (WENG et al., 2008; KOPEC et al., 2015).

Recently, several publications have demonstrated the high potential of agronomic biofortification in increasing the contents of Se, I, and Zn in crops such as rice (NAEEM et al., 2021; PROM-U-THAI et al., 2020), wheat (ZOU et al., 2019; CAKMAK et al., 2020), lettuce (SAHIN, 2020), and potato (MAO et al., 2014) grown in the field. However, the success of agronomic biofortification depends on a series of factors such as plant species and cultivars, the form of the element, type of application (via soil or foliar), type of soil, environmental conditions, and others.

Due to this concern, the number of studies related to the biofortification of food crops has been increasing and sweet potatoes (*Ipomoea batatas* L.) are among those with a high potential for biofortification. Naturally, some sweet potato cultivars are excellent sources of vitamin A as well as a good source of vitamins B5 and B6 (HORODYSKA et al., 2021). However, Zn concentration in the edible roots of sweet potatoes is the lowest among all staple food crops (ADETOLA et al., 2020).

Selenium contents found in plants are directly related to Se availability in the soil (LOPES et al., 2017) where the plant is cultivated. The use of soil Se fertilizers can increase Se concentration in grains, fruits, and vegetables by several times (LIU et al., 2011; YIN; LI, 2011), but studies in field conditions are required to better define the Se rates to be applied since the limit between essentiality and toxicity of Se is very narrow (VAN HOEWYK, 2013). Most Brazilian soils are Se deficient, with Se contents ranging from 0.01 to 0.2 mg kg^{-1} (CARVALHO et al., 2018; LOPES et al., 2017; MIRLEAN et al., 2018; REIS et al., 2017; SILVA JUNIOR et al., 2017). In this context, it has to be mentioned that the Brazilian Ministry of Agriculture, Livestock, and Supply included Se as a possible micronutrient to be added to Brazilian fertilizers, according to the Normative 46/2016 (BRAZIL, 2016). This normative establishes the minimum contents of Se added to fertilizers destined for agriculture in Brazil.

Few countries have well-documented regulations for the use of Se-fertilizers in crops, with Finland being a great example (ALFTHAN et al., 2015), but this country has soils with distinct characteristics from those found in tropical environments. Thus, studies in tropical regions are of great relevance. Selenium intake by population through the use of fertilizers containing Se, might be increased since this has been a satisfactory strategy to be performed in countries where the natural Se content or availability in soils is low, such as Brazil.

Selenium can undergo several interactions in the environment (SCHIAVON et al., 2020), with its sorptive behavior being different among soils. Inorganic forms of Se, *i.e.*, selenate and selenite, may compete with organic acids as well as with other anions, such as phosphate and sulfate for soil adsorption sites (NATASHA et al., 2018). This fact is relevant for agroecosystems, particularly, for tropical soils taking into account that these soils usually receive high amounts of phosphate and sulfate, which come up from agricultural products applied on soils, such as fertilizers and gypsum (Lopes et al., 2017). Studies demonstrate a series of benefits in the use of commercial fertilizers enriched with Se such as coated MAP (ARAUJO et al., 2022; SILVA et al., 2022) and urea (RAMKISSOON et al., 2019; FÉLIX et al., 2023). In addition to providing nitrogen and phosphorus more efficiently, these fertilizers can also provide other elements to plants, such as Se.

Unless the efficacy of Se fertilizers can be improved, there is a need to be reapplied over time to keep Se contents in food crops in adequate amounts, and farmers need to be carefully instructed on rates and methods of application (WU et al., 2015). In order to neutralize the effects of soil constituents on the applied forms of Se, another efficient strategy for food biofortification is via foliar application. Studies have compared the efficiency of applying Se by different methods and results have shown that, although both are effective in enhancing plant Se concentrations, foliar fertilization is more efficient than soil Se application (BOLDRIN et al., 2013; MAO et al., 2014; ZOU et al., 2019; LESSA et al., 2020).

The greater efficiency of foliar-applied fertilizers may be attributed to the rapid uptake and assimilation due to application at a later growth stage, less influence of the root-to-shoot ratio on translocation to the edible parts of crops, and no interactions with soil solid constituents (RAMKISSOON et al., 2019). On average, only 12% of soil-applied Se fertilizers are taken up by plants; most Se applied is retained and immobilized in the soil (BROADLEY, 2010), with very little residual value for subsequent crops (MATHERS, 2017).

So far, few studies have studied the agronomic biofortification of sweet potatoes with Se (EL-RAMADY et al., 2014; SONG et al., 2018; GAO et al., 2021) and, to the best of our knowledge, no studies with this focus involving sweet potatoes grown in tropical soils were found in the literature. In this context, this study evaluated different strategies for agronomic biofortification of sweet potato with Se and iodine (I), aiming to: i) assess the biofortification potential of Se in the presence or absence of I addition, in response to increasing Se rates applied as foliar spray; ii) evaluate Se(VI) adsorption capacity in a tropical soil and its further desorption by phosphorus and nitrogen (from pure salts or as MAP/urea); and iii) assess biofortification of Se by applying increasing Se rates in the soil together MAP and/or urea fertilizers (Se-enriched fertilizers).

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SECOND PART – ARTICLES

ARTICLE 1: COMBINED BIOFORTIFICATION OF SWEET POTATO WITH FOLIAR APPLICATION OF SELENIUM, IODINE, AND ZINC

This article was prepared in line with the guidelines of the journal: Plants, which will be submitted to.

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Abstract

Agronomic biofortification is an efficient strategy for overcoming the nutritional deficiency of food by increasing human-essential nutrient contents in edible parts of plants that are grown in regions where soils are naturally poor in these nutrients. Selenium (Se), iodine (I), and zinc (Zn) are examples of these essential elements, and biofortification studies assessing the effects of foliar applications of Se, I, and Zn in sweet potatoes are of great relevance. This study aimed to assess under field conditions the agronomic biofortification potential of sweet potato plants with Se, I, and Zn for the first time, by evaluating the contents of these elements in its edible part in response to foliar application of increasing Se rates with or without I and also by the simultaneous addition of Se, I, and Zn. Selenium was applied in different rates (0, 50, 100, and 200 g ha^{-1}) in the absence or the presence of I (500 g ha^{-1}), with an additional treatment consisting of the higher rates of Se+I and 2.5 kg ha^{-1} of zinc (Zn). All micronutrients were applied in a fertilizer, potassium nitrate. Results have shown that Se contents in sweet potato shoots and roots increased upon increasing Se rates applied, irrespectively, in general, of the I addition. Moreover, the addition of Zn was relevant for enhancing Se contents, since the simultaneous application of Se, I, and Zn resulted in much higher Se contents determined in sweet potato. The highest I content (0.2 mg kg^{-1}, accounting for 7.7 µg person^{-1} day^{-1}) was observed at the lowest Se rate (50 g ha^{-1}) and applying higher Se rates reduced I contents compared with the control. This treatment (50g ha^{-1} of Se + I) was highlighted and could provide 19.1 µg person^{-1} day^{-1} of Se (27.3% of the RDA for Se), and 7.7 µg person^{-1} day^{-1} (5.15% of the RDA for I). Based on the consumption of 156 g of fresh sweet potato roots, maximal contribution of the tripple nutrient biofortification (Se+I+Zn) would be 78 µg person^{-1} day^{-1} of Se, which is 111% of the recommended daily Se intake for an adult.

Keywords: *Ipomoea batatas* L. Selenate. Iodate. Hidden hunger. Beneficial element. Tropical soil.

1 INTRODUCTION

Malnutrition affects more than one-quarter of the population globally, with this health problem referring to the nutritional deficiency of some micronutrients, including Se (selenium), I (iodine), and Zn (zinc). Although Se and I are not considered essential to plants, their importance has already been recognized as beneficial nutrients, due to their important role in various biological processes that are crucial for plants, animals, and humans' growth and development (Hatfield et al., 2014; Kiferle et al., 2021; Brown et al., 2022). For humans, the main cause of micronutrients deficiency is the poor of accessing to a diversified diet, capable of supplying adequate amounts of nutrients. The main cause of lack of I, Zn or Se in the diet is the low micronutrient contents found in agricultural crops that are grown in soils with low availability of these nutrients for uptake by the plant, as in tropical soils.

Soil deficiencies in Se and Zn are directly related to the human deficiency incidence of these elements worldwide (Lopes et al., 2017; Cakmak and Kutman, 2018). It has been estimated that approximately one billion people are affected by Se deficiency worldwide (Schiavon and Pilon-Smits, 2017). Selenium consumption is often associated with the prevention of health problems such as cardiovascular diseases, Keshan's disease, Kashin-Beck, viral infections, type II diabetes, hypothyroidism, low immunity, male infertility, and certain types of cancer (Saha et al., 2017). Meanwhile, Zn acts in the human body by attenuating the effect of viruses and bacteria, with this element being involved in the improvement of the immune system (Read et al., 2019; Skalny et al., 2020).

Iodine is also essential to humans and its deficiency leads to health problems related to the biosynthesis of thyroid hormones (Köhrle, 2023). The prevalence of I deficiency in humans worldwide is lowered by its addition into salt (NaCl) (Zimmermann and Andersson, 2021). Due to both Se and I are related to the thyroid metabolism and health, it is reported that Se deficiency may endanger the effectiveness of salt iodization programs (Gashu et al., 2016). However, salt consumption has become a problem for people suffering from hypertension, and new strategies must be established to supply this element to humans in adequate amounts. The recommended daily allowance (RDA) of I for humans ranges between 150–200 µg day^{-1} (Lawson et al., 2015), while the requirements of Se and Zn of an adult are 60-70 µg day^{-1} (Kipp et al., 2015) and 8-11 mg day^{-1} (Maggini, et al., 2018), respectively.

Most soils in the Brazilian Cerrado biome are deficient in Se, with Se contents ranging from 0.01 to 0.2 mg Se kg^{-1} (Carvalho et al., 2018). Zinc contents in tropical soils are also low (Lopes and Guilherme, 2016). Therefore, studies evaluating Se application strategies for the agronomic biofortification of food crops cultivated in tropical soils are of great relevance for increasing Se contents in edible parts (Lopes et al., 2017), and the addition of Zn together Se may be interesting to increase the accumulation of Se in plants since Zn is involved in proteins synthesis, being accumulated as selenoproteins in plants (Persson, et al., 2016; Roda et al., 2020; Bañuelos, et al., 2022).

Iodine concentration in rain water is the most available source of I for plants. In Brazil, very low I concentrations in drinking water derived from reservoirs have been found. In the summer season, mean I concentration in water of five Brazilian macro regions reached a maximum value of 0-13.04 µg L^{-1} (0-0.1 micromol L^{-1}), with even lower I concentrations being detected in the fall (0-2.45 µg L^{-1}) and in the winter (0.69-4.92 µg L^{-1}) seasons (Pinto et al., 2022). Additionally, since I has a high affinity to sorb to dissolved organic carbon, high Fe-oxide contents may increase the fixation potential, hence decreasing I release from soils to aquatic systems in tropical soils (Balzer et al., 2020).

Researches assessing the simultaneous soil and/or foliar applications of I and Se have been carried out in several food crops such as apples and pears (Budke et al., 2021), kohlrabi (Golob et al., 2020b), Indian mustard (Golubkina et al., 2018), and carrots (Smolén et al., 2019). According to Lyons et al. (2018), the potential benefits of co-applying Se and I, along fertilizer and pesticide application, could make biofortification commercially viable for farmers. Also, simultaneous application of Se, I, and Zn via foliar sprays on rice and wheat has shown to be effective under field conditions (Mao et al., 2014; Cakmak et al., 2017; Zou et al., 2019; Prom-u-thai et al., 2020; Sahin, 2020).

The effectiveness of agronomic biofortification depends on several aspects, including chemical sources (inorganic and/or organic) and application methods (foliar and/or via soil), with foliar application being more efficient than soil addition for increasing Se content in crops edible part (Boldrin et al., 2013; Lessa et al., 2020). This happens due to the adsorption process occurring in soils, mainly in tropical soils (Mouta et al., 2008; Lessa et al., 2016; Araujo et al., 2019).

Agronomic biofortification of Se has been successfully done with food crops grown in tropical soils, as wheat (Lara et al., 2019), rice (Boldrin et al., 2013; Lessa et al., 2020), common bean (Ravello et al., 2021), potato (de Oliveira et al., 2019), carrot (de Oliveira et al., 2018), and strawberry (Santiago et al., 2018). However, to the best of our knowledge, there were no studies conducted in tropical soils focusing on biofortification of sweet potatoes (*Ipomoea batatas* L.) with Se, I, and Zn. Sweet potato is a naturally nutritious source of energy due to its high starch content, and low glycemic carbohydrates, combined with presence of fibers, and nutrients (iron, magnesium, and potassium), vitamins - such as complex A, C, and B6, and bioactive plant metabolites, such as carotenoids - especially β-carotene (provitamin A), anthocyanins, and other phenolic compounds (Ramos et al., 2021; Frond et al., 2019). Due to the beneficial nutrient-profile, and excellent taste, sweet potato consumption is an attractive alternative for carbohydrate-rich foods like potato or wheat in developed countries, being also a significant source of vitamin A and energy in countries of Asia, Africa, and Latin America (Cartabiano-Leite et al., 2020; Stathers et al., 2018). Thus, studies evaluating the enrichment of sweet potatoes with these micronutrients (*i.e.,* Se, I, and Zn) are required, being very relevant in the context of food security.

Therefore, this study aimed to assess under field conditions the biofortification potential of sweet potato plants with Se, I, and Zn, by evaluating the contents of these elements in its edible part in response to foliar application of a commonly applied foliar fertilizer source (potassium nitrate) containing increasing Se rates with or without I and also by the simultaneous addition of Se, I, and Zn. In addition, the growth and development of sweet potato plants after applying the foliar fertilizer with the different rates and combinations of micronutrients were assessed.

2 MATERIAL AND METHODS

2.1 Experimental area and treatments

This study was performed with an early sweet potato cultivar (var. Ligeirinha). The experiment was carried out under field conditions at 3W Agronegócios farm located in Carrancas, Minas Gerais State, Brazil (21°32′52” S; 44°43′24” W). The average annual precipitation in the cultivated field was 1636 mm, and the average annual temperature was 18.7°C, with mild and humid summers with a maximum average temperature of 25.2ºC and cold and dry winters, with a minimum average temperature of 11.5ºC.

The experiment was established in a randomized block design, composed of nine treatments with 5 replicates, totaling 45 experimental plots with 24 m^2. Each plot consisted of 5 m long by 4.8 m wide (4 rows, with row-spacing at 1.2 m), with the 2 central sweet potato lines (excluding 1 m in each extremity) being used as useful area for evaluating the variables measured in this study. Planting was made with 4 sweet potato stem-segments (0.25 m to 0.3 m) per linear meter and the base fertilizer was applied at planting with 150 kg ha^{-1} of monoammonium phosphate (MAP = 10.5% N and 52% P_2O_5). Twenty-five days after transplanting, additional nitrogen (N) and potassium (K) were top-dressed at the rate of 250 kg ha^{-1} of urea and 330 kg ha^{-1} of potassium chloride (KCl), respectively. Total amounts of nutrients applied were 131 kg N ha^{-1}, 78 kg P_2O_5 ha^{-1}, and 198 kg K_2O ha^{-1}, based on soil testing and as commonly performed in the farm for achieving high crop yield.

Selenium was applied as sodium selenate (Na_2SeO_4, Sigma-Aldrich, Saint Louis, MO, USA) in the following Se rates: 0, 50, 100, and 200 g ha^{-1}. These Se rates were applied as foliar sprays with or without the application of 0.5 kg ha^{-1} of I, applied as potassium iodate (KIO_3, Synth, Diadema, Sao Paulo, Brazil). Also, an additional treatment applying 0.5 kg I ha^{-1} + 200 g Se ha^{-1} + 2.5 kg Zn ha^{-1} (as $ZnSO_4.7H_2O$, Êxodo Científica, Hortolândia, Sao Paulo, Brazil) was evaluated. To increase the uptake and translocation of the micronutrients from the spray solution, Se, I, and Zn salts were diluted in plastic bottles with deionized water with 2% w/w of potassium nitrate (KNO_3) (Dripsol®, SQM Vitas), and subsequently 0.5% v/v of surfactant (Assist®, BASF) was added. The control treatment received only deionized water, surfactant, and KNO_3. The equivalent volume of solution applied each time was 200 L ha^{-1}. The solution was applied using a carbon dioxide pressurized pump, with a conical spray nozzle spraying the solution on the sweet potato leaves. The rates of Se, I, and Zn, in kg ha^{-1}

as aforementioned, were split over three applications, which were performed at 49, 80, and 109 days after transplanting. Leaf samples were collected ten days after each application period for further analysis.

Physical and chemical characteristics of the soil in the experimental area before treatment applications were determined according to the methodology proposed by the Brazilian Agricultural Research Corporation (EMBRAPA) (Teixeira et al., 2017), and the main soil characteristics were as follows: pH (H_2O) = 6.55; pH ($CaCl_2$) = 5.95; soil organic matter = 39.5 g kg^{-1}; P (Mehlich-1) = 1.15 mg dm^{-3}; P-rem = 24.6 mg L^{-1}; P-resin = 26.9 mg dm^{-3}; K = 245.50 mg dm^{-3}; S = 13.9 mg dm^{-3}; Ca = 3.90 $cmol_c$ dm^{-3}; Mg = 1.60 $cmol_c$ dm^{-3}; B = 0.44 mg dm^{-3}; Cu = 0.55 mg dm^{-3}; Fe = 31.9 mg dm^{-3}; Mn = 10.9 mg dm^{-3}; Zn = 0.95 mg dm^{-3}; Al = 0.10 $cmol_c$ kg^{-1}; H+Al = 1.95 $cmol_c$ kg^{-1}; SB = 6.15 $cmol_c$ kg^{-1}; CEC = 8.10 $cmol_c$ kg^{-1}; clay = 220 g kg^{-1}; silt = 110 g kg^{-1}; and sand = 670 g kg^{-1}.

2.2 Yield and dry matter production of shoot and root

Sweet potato plants were grown for 159 days. During the harvesting, shoots of all plots were collected and their fresh weight was recorded. Then, shoot samples from each experimental plot were transported to the laboratory, where all samples were dried in an oven with forced air circulation at 65°C for 72 h until constant weight. The dry weigh of shoot was also recorded. The potato (root) harvested was first classified as commercial sweet potatoes according to Azevedo et al. (2002) and Huáman (1992) and those that did not meet the commercial quality were discarded. Similarly, to what was done with the shoots, fresh sweet potato was also weighed, peeled, and dried in an oven with forced air circulation at 65°C for 72 hours until constant weight and weighted again for recording the dry matter of potato roots. After that, both materials (shoot and root/sweet potato) were ground in a 1.0 mm Willey mill for further analysis.

2.3 Selenium, iodine, macronutrient, and micronutrient contents in biofortified sweet potato

For determining the contents of Se, I, and other elements in sweet potato (leaves, shoot, and peeled root), plant materials (ground samples) were shipped to the laboratory of the University of Nottingham. Then, they were first submitted to acid digestion using concentrated HNO_3 and closed vessels in a microwave oven (Mars 5CEM Corporation, Matthews, EUA), according to the 3051A method of the United States Environmental

Protection Agency – USEPA (USEPA 2007). Then, extracts were analyzed by atomic absorption spectroscopy with electro-thermal atomization in a graphite furnace (Perkin Elmer, model AA-analyst 800, Midland, Canada). Total N contents were also determined by the Kjeldahl method.

For quality assurance and control, two samples of standard reference material (Wheat flour - NIST, National Institute of Standards and Technology, Gaithersburg, USA - with 1.14 mg Se kg^{-1} and 11.61 mg Zn kg^{-1} - and Hay powder – BCR, Community Bureau of Reference, European Commission - with 0.167 mg I kg^{-1}) were included in each batch of digestion. The mean recoveries for Se, Zn, and I found using these reference materials were 96, 95, and 87% respectively.

The amount of Se or I from shoot, in g ha^{-1}, that remained in the field after sweet potato harvesting, termed here as $M_{residual}$ (micronutrient residual), was estimated considering Se or I contents determined in shoot (g kg^{-1} DW) and total production of dry matter of shoot estimated as aforementioned, using the following equation:

$$M_{residual}\ (\text{g ha}^{-1}) = [(\text{M in DM (mg kg}^{-1}) * \text{DM of shoot (kg ha}^{-1})] / 1000$$

Moreover, the fraction of the applied Se and I that was incorporated in sweet potato roots and then removed (M removal) was calculated as follows:

$$M_{removal}\ (\%) = (((M_{treatment} - M_{control}) * Y)) / M_{rate}) * 100$$

where: $M_{removal}$ (%) is the use efficiency of Se or I rates applied in the leaves by harvested sweet potato roots; $M_{treatment}$ (g kg^{-1}) = Se or I contents in sweet potato from plants grown in treatments that received the application of these elements; $M_{control}$ (g kg^{-1}) = Se contents in sweet potato roots from plants grown in treatments without the application of these elements; Y = Yield of sweet potato, in dry weight (kg ha^{-1}); M_{rate} (g ha^{-1}) = Se or I rates applied in the treatment.

According to Horodyska et al. (2021), the average daily consumption of fresh sweet potatoes by Brazilian consumers is 156 g person^{-1} day^{-1}. Based on this information and taking into account the Se, I, and Zn contents determined on the dried powdered peeled sweet potato of the present study, we have also calculated the possible intake of micronutrients (M_{intake}) from the consumption of these biofortified roots, using the equation below:

$$M_{intake} = M_{treatment} * DM * C$$

where: M_{intake} (µg person^{-1} day^{-1}) is the daily Se, I or Zn intake estimation per person, $M_{treatment}$ (µg kg^{-1}) is the Se, I or Zn contents in peeled sweet potato verified for the studied treatments, DM (%) is the percentage of dry matter of the harvested sweet potato (25%), and C (kg person^{-1} day^{-1}) is the mean consumption of fresh sweet potato per person from Brazilian consumers (Horodyska et al., 2021).

2.4 Statistical analysis and data processing

Data were submitted to analysis of variance and treatment means were compared by Scott Knott's test ($p \leq 0.05$) using the Sisvar software, version 5.6 (Ferreira, 2011). The additional treatment with Zn was compared by Dunnett's test ($p \leq 0.05$) against all treatments and included in the graphs through dashed lines. Graphics were performed using the Sigma Plot software, version 12.0 (Systat Software Chicago, IL, USA).

3 RESULTS

3.1 Production of shoot dry mass and sweet potato dry yield

Shoot dry mass (SDM) and sweet potato dry yield (Fig. 1) were influenced ($p \leq 0.05$) by the interaction between the studied factors (Se rates and the presence or absence of I). There was no significant effect of the treatments on SDM (Fig. 1a) with an average SDM of 8.86 t ha^{-1}. In contrast, the yield of dry sweet potato roots varied slight among treatments (Fig. 1b), with the highest values being 9.87 and 9.57 t ha^{-1}, resulting from 50 g ha^{-1} of Se were applied in the presence of I, and in the control treatment (without Se and I). The application of Se at 50 g ha^{-1}, in the presence of I at 0.5 kg I ha^{-1}, resulted in the highest sweet potato dry yield. In the absence of I, application of Se in all concentrations reduced the yield, compared to the control treatment without Se or I (Fig. 1b). The presence of I reduced the sweet potato dry yield when plants were also treated with 0 or 200 g ha^{-1} of Se, compared with Se treatments without I.

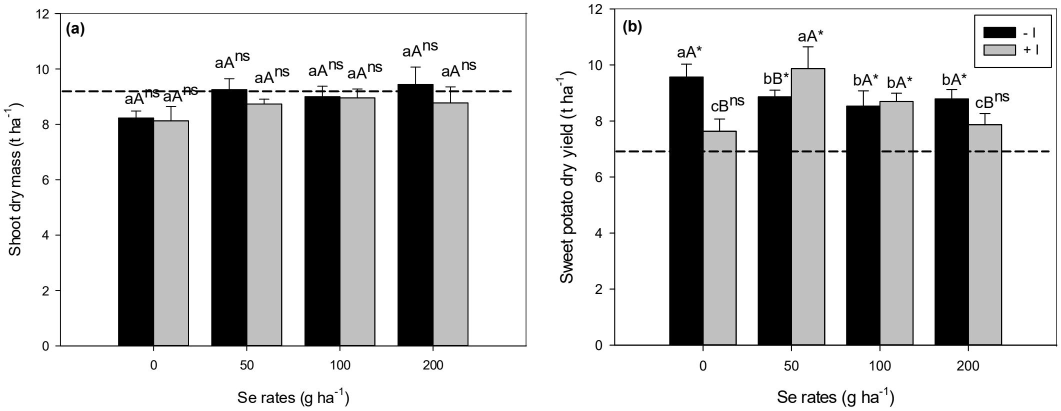

Fig. 1. Production of shoot dry mass (a) and dry yield (b) of sweet potato. Uppercase letters compare the means with or without iodine within each Se rate and lowercase letters compare the means of Se rates at each iodine condition (with or without), according to Scott-Knott's test ($p \leq 0.05$). The dashed line refers to the value found for the additional treatment (Se at 200 g ha^{-1} + I at 0.5 kg ha^{-1} + Zn at 2,5 kg ha^{-1}). "ns" and "*" indicate non-significant and significant differences of the additional treatment compared with the other bars by the Dunnett's test ($p \leq 0.05$). Vertical bars indicate the standard error of average values ($n = 5$).

3.2 Selenium contents in leaves, shoot, and root

Selenium content in sweet potato leaves, collected in the middle third of the sweet potato plants, in healthy and completely expanded leaves, 10 days after each application of solution containing Se, I, and Zn, increased upon increasing Se rates (Fig. 2), with the highest Se content being observed when Se was added at 200 g ha^{-1}. Co-application of Se with I affected Se contents in the leaves after the first application (Fig. 2a) only when Se was added at the highest rate (200 g ha^{-1}). In this case, additional I application reduced Se contents in leaves compared to the same Se rate without I. This also happened in the second leaf collection (Fig. 2b) when I was applied with either 100 or 200 g ha^{-1} of Se, but there was no interaction between I on Se contents in leaves collected after the third application (Fig. 2c), 109 days after transplanting. Interestingly, Se contents in leaves from all collection times (after 1st, 2nd, and 3rd application) verified for the additional treatment, where the highest Se rate (200 g ha^{-1}) was applied in combination with I (0.5 kg ha^{-1}) and Zn (2.5 kg ha^{-1}) (Fig. 2), were greater than those found after the application of 200 g ha^{-1} of Se (with or without I), except for leaves collected after the second foliar application and when 200 g ha^{-1} of Se was added in the absence of I, where no statistical differences were observed (Fig. 2b).

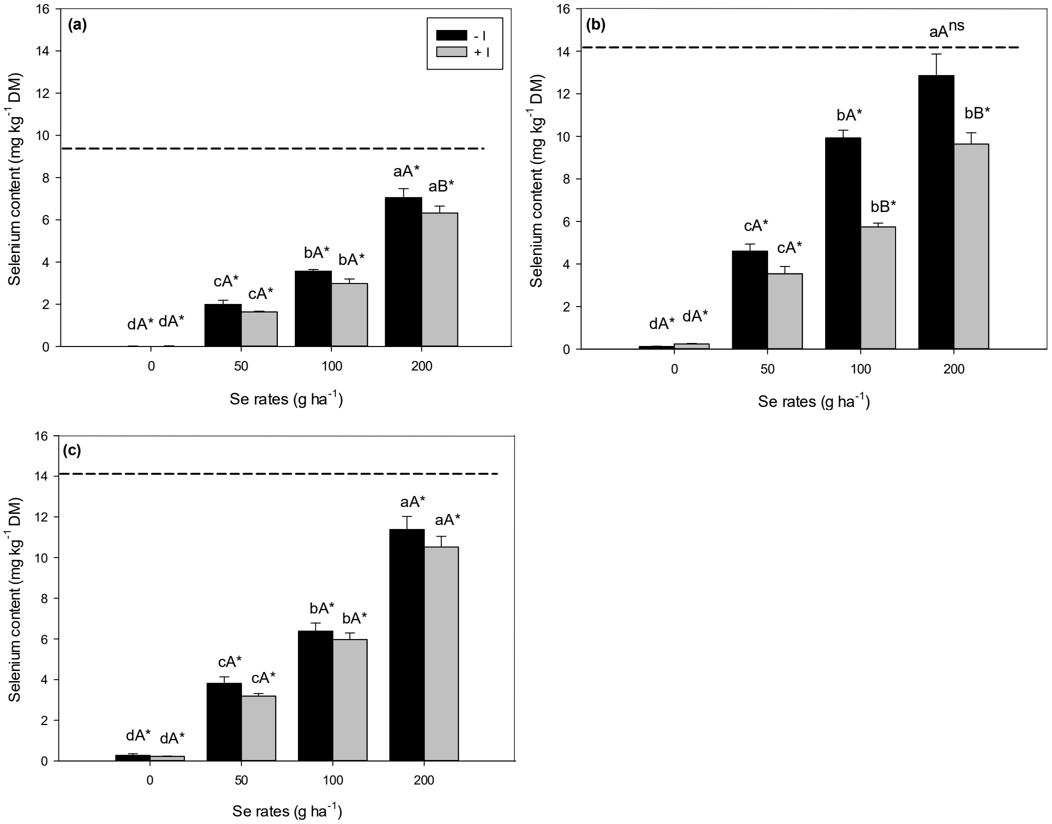

Fig. 2. Selenium content in sweet potato leaves after the first (a), second (b), and third (c) application of solutions containing Se, I, and/or Zn. Uppercase letters compare the means with or without iodine within each Se rate and lowercase letters compare the means of Se rates at each iodine condition (with or without), according to Scott-Knott's test ($p \leq 0.05$). The dashed line refers to the value found for the additional treatment (Se at 200 g ha^{-1} + I at 0.5 kg ha^{-1} + Zn at 2,5 kg ha^{-1}). "ns" and "*" indicate non-significant and significant differences of the additional treatment compared with the other bars by the Dunnett's test ($p \leq 0.05$). Vertical bars indicate the standard error of average values (n = 5).

Similar to what was reported for sweet potato leaves, Se contents in sweet potato shoot and root increased with the increase of Se rates (Fig. 3), ranging from 0 to 3.18 mg kg^{-1} and from 0 to 1.48 mg kg^{-1}, respectively. The presence of I in the foliar application of Se did not affect Se contents in sweet potato shoot and root, except for root (sweet potato) in plants treated with 100 g Se ha^{-1}, where higher Se contents were verified in the absence of I (Fig. 3b). As aforementioned for Se contents in leaves, the greater Se contents in sweet potato shoots and roots were observed for the additional treatment containing Zn (Fig. 3a and 3b), reaching, respectively values of 4.5 and 2.0 mg Se kg $^{-1}$. The presence of Zn in plants treated with 200 g Se ha^{-1} increased Se contents in sweet potato shoots and roots by approximately 35 and 36 % in treatments with I, and by 49 e 37% in the absence of I.

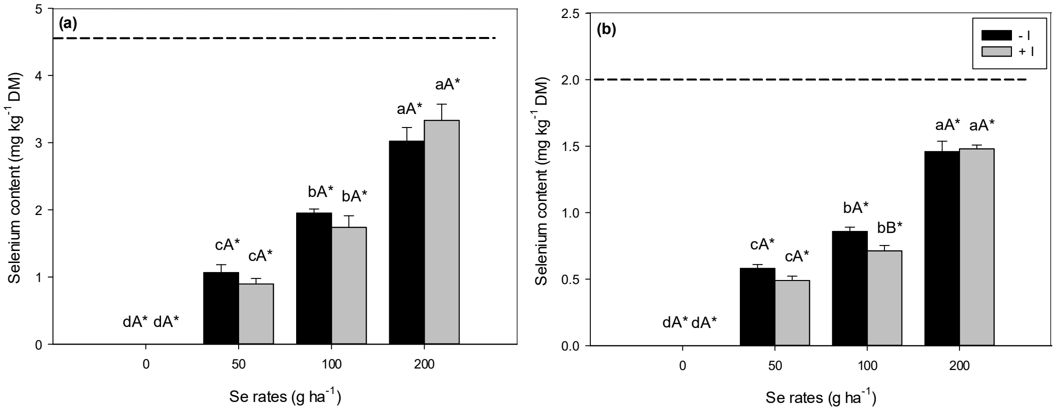

Fig. 3. Selenium content in sweet potato shoots (a) and roots (b) after harvest. Uppercase letters compare the means with or without iodine within each Se rate and lowercase letters compare the means of Se rates at each iodine condition (with or without), according to Scott-Knott's test ($p \leq 0.05$). The dashed line refers to the value found for the additional treatment (Se at 200 g ha^{-1} + I at 0.5 kg ha^{-1} + Zn at 2,5 kg ha^{-1}). "ns" and "*" indicate non-significant and significant differences of the additional treatment compared with the other bars by the Dunnett's test ($p \leq 0.05$). Vertical bars indicate the standard error of average values (n = 5).

3.3 Iodine contents in leaves, shoot, and root

The interaction between Se rates and I was relevant in the leaves collected 10 days after each treatment application (Fig. 4). Significant effects of Se rates on I contents determined in leaves were observed. In the first leaf collection, I leaf content decreased by co-application in the highest Se rates (100 and 200 g Se ha^{-1}). Also, in the second and third collections, higher I contents were found with the lowest Se rate (50 and 100 g Se ha^{-1} for the second collection and 50 g Se ha^{-1} for the third one). Comparing the collection periods within each Se rates, different I contents were found, with much higher I contents being verified in the second collection period.

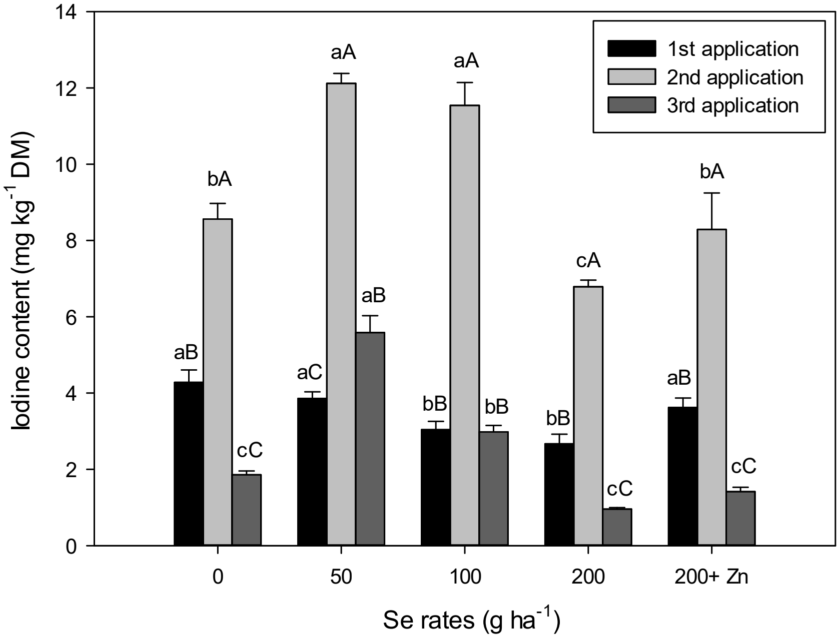

Fig. 4. Iodine content in sweet potato leaves after the first (a), second (b), and third application (c) of solutions containing Se, I, and/or Zn. Uppercase letters compare the means of iodine contents within each Se rate and lowercase letters compare the means of iodine contents among Se rates within the same collection time, according to Scott-Knott's test ($p \leq 0.05$). Vertical bars indicate the standard error of average values (n = 5).

Iodine contents in sweet potato shoots and roots, in treatments that received foliar addition of I, are presented in Figure 5. Iodine contents found in the shoots were greater when plants received 50 and 100 g Se ha^{-1} (Fig. 5a). In the roots (peeled sweet potato), the highest I content was 0.2 mg kg^{-1}, which was found when 50 g ha^{-1} of Se was applied, with I contents being afterwards reduced upon increasing Se rates (Fig. 5b). Thus, the lowest I content was found when 200 g ha^{-1} of Se was applied, and the presence of Zn in the applied solution (Se + I + Zn) increased I contents to values comparable to what was verified for the treatment that received 100 g ha^{-1} of Se. Iodine content in the treatment that received only the application of I (without Se application) was only lower than that verified when 50 g ha^{-1} of Se was applied.

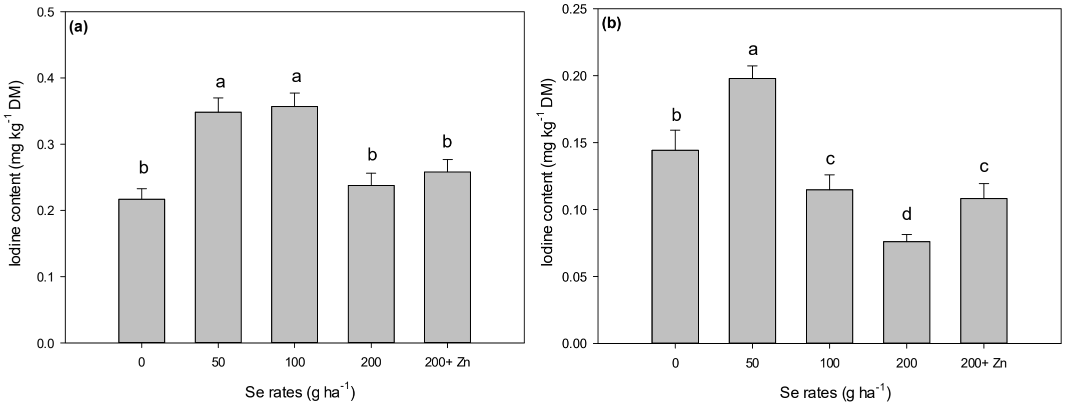

Fig. 5. Iodine content in sweet potato shoot (a) and root (b) after harvesting. Lowercase letters compare the means of iodine contents among Se rates/treatments, according to Scott-Knott's test ($p \leq 0.05$). Vertical bars indicate the standard error of average values ($n = 5$).

3.4 Removal of selenium and iodine

The percentage of Se removed by sweet potato roots ranged from 5.81% to 10.29% (Fig 6a), while removal of I showed much lower values, ranging from 0.12% to 0.4% (Fig. 6b). The treatments that received the lowest Se rate (50 g ha^{-1}) showed higher percentages of Se and I removal by roots. Selenium removal did not differ statistically as a function of I treatments.

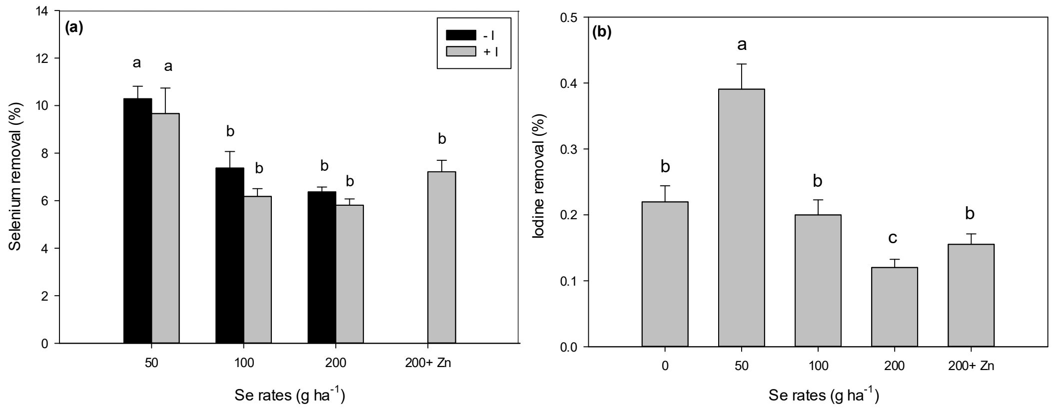

Fig. 6. Selenium (a) and iodine (b) removal (%) in sweet potato roots after harvesting. Lowercase letters compare Se and I removal among treatments, according to Scott-Knott's test ($p \leq 0.05$). Vertical bars indicate the standard error of average values ($n = 5$).

3.5 Estimation of daily intake of Se and I by consuming biofortified sweet potato

Based on the average consumption of sweet potato by Brazilian consumers (Horodyska et al., 2021), which is 156 g person^{-1} day^{-1} on a fresh-weight basis (FW), Figure 7 shows the estimation of Se and I intake considering the consumption of the biofortified sweet potato produced in this present study. Foliar application of I within each Se rate did not affect Se intake, except at 100 g Se ha^{-1} (Fig. 7a), where the application of Se alone would moderately improve Se intake. Selenium intake enhanced upon enhancing Se rates applied as a foliar spray. On the contrary, I intake was higher when 50 g ha^{-1} of Se was applied and decreased upon increasing Se rates, compared with the control (without Se) (Fig. 7b). As highlighted in the results of Se contents, the combined application of Se+I+Zn (additional treatment - 200 g ha^{-1} of Se + 0.5 kg ha^{-1} of I, and 2.5 kg ha^{-1} of Zn) resulted in higher Se contents in sweet potato root. As a consequence, the higher Se intake estimation was obtained in this treatment, accounting for 78 µg person^{-1} day $^{-1}$ (111% of the RDA, which is 70 µg person^{-1} day $^{-1}$ - Kipp et al., 2015). The highest I intake was observed when this element was co-applied with Se at 50 g Se ha^{-1}. This treatment (combined addition of I at 0.5 kg ha^{-1} with 50 g ha^{-1} of Se) might contribute to the intake of approximately 7.7 µg person^{-1} day $^{-1}$ of I (5.15% of the RDA, which is 150 µg person^{-1} day $^{-1}$ - Lawson et al., 2015), and 19.1 µg person^{-1} day^{-1} of Se (27.3% of the RDA).

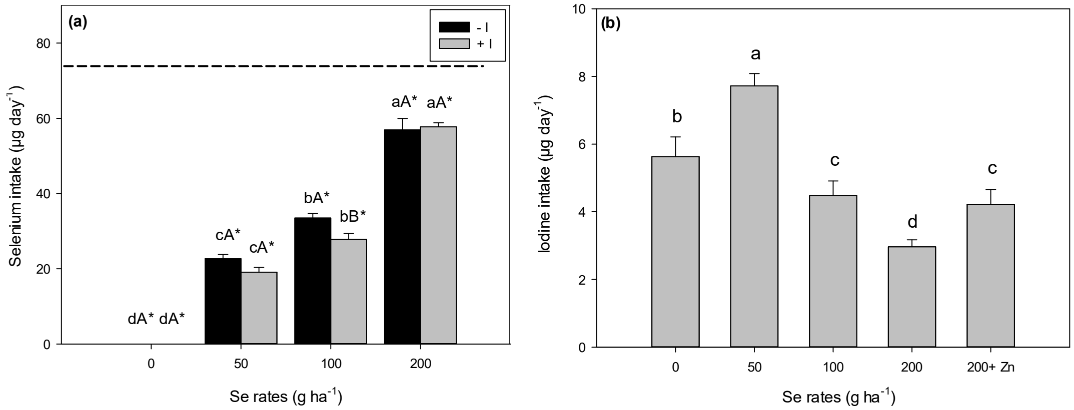

Fig. 7. Estimation of potential daily Se (a) and I (b) intake per person by consuming the biofortified sweet potato produced in this present study (dry matter of sweet potato = 75%). Uppercase letters compare the means of iodine foliar spray at each Se rate, and lowercase letters compare the means of Se rates at each iodine condition, according to Scott-Knott's test ($p \leq 0.05$). The dashed line refers to the value found for the additional treatment (Se at 200 g ha^{-1} + I at 0.5 kg ha^{-1} + Zn at 2,5 kg ha^{-1}). "ns" and "*" indicate non-significant and significant differences of the additional treatment compared with the other bars by the Dunnett's test ($p \leq 0.05$). Vertical bars indicate the standard error of average values (n = 5).

3.6 Macronutrients and micronutrients in sweet potato root

The tested treatments affected contents of macronutrients and micronutrients determined in sweet potato roots (Tables 1 and 2). In addition to increasing Se contents (as aforementioned), the addition of Zn in the additional treatment also increased the contents of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and sulfur (S) in sweet potato roots (Table 1). Application of I with Se increased nitrogen (N) and (K) contents of the roots at all Se rates. Differently from what was verified for macronutrients, the highest boron (B) content was observed in treatment that received 50 g Se ha^{-1} with I. Copper (Cu) contents showed lower values in all treatments that received Se without I. Iron (Fe) and magnesium (Mn) expressed their greatest contents in treatments that received 100 and 200 g

Se ha^{-1} in the presence of I. Irrespectively of the I addition, the higher Se rates decreased and increased molybdenum (Mo) and nickel (Ni) contents, compared with the control, respectively.

Applying 2.5 kg Zn ha^{-1} in combination with 200 g Se ha^{-1} + I (500 g ha^{-1}), Zn contents in sweet potato roots increased by approximately 40.3%, when compared with the control. Taking into account the consumption of 156 g person^{-1} day^{-1} (Horodyska et al., 2021), consuming the sweet potato roots from this treatment with Zn, a person may ingest 0.3 mg day^{-1}, which corresponds to 3.75% of the recommended daily Zn requirements for an adult (Maggini et al., 2018).

Table 1. Mean contents of macronutrients (g kg^{-1}) in the dry matter of sweet potato roots

Treatment	N	P	K	Ca	Mg	S
	g kg^{-1}					
0 g Se ha^{-1}	7.58 ± 0.22 Aa*	1.34 ± 0.05 Aa*	13.56 ± 0.27 Aa*	0.58 ± 0.03 Aa*	0.39 ± 0.02 Ab*	0.54 ± 0.03 Ab*
50 g Se ha^{-1}	6.63 ± 0.13 Ba*	1.46 ± 0.04 Ba*	14.02 ± 0.50 Ba*	0.58 ± 0.03 Aa*	0.42 ± 0.02 Ab*	0.62 ± 0.01 Aa*
100 g Se ha^{-1}	7.10 ± 0.36 Ba*	1.47 ± 0.04 Aa*	13.90 ± 0.32 Ba*	0.64 ± 0.02 Ba*	0.40 ± 0.01 Ab*	0.61 ± 0.02 Aa*
200 g Se ha^{-1}	7.00 ± 0.10 Ba*	1.52 ± 0.03 Aa*	13.91 ± 0.14 Ba*	0.66 ± 0.02 Aa*	0.43 ± 0.01 Aa^{ns}	0.58 ± 0.02 Aa*
0 g Se ha^{-1} + I	7.64 ± 0.35 Ab*	1.44 ± 0.06 Ab*	13.23 ± 0.56 Ab*	0.56 ± 0.02 Ab*	0.42 ± 0.01 Ab*	0.53 ± 0.03 Ab*
50 g Se ha^{-1} + I	8.95 ± 0.27 Aa^{ns}	1.61 ± 0.11 Aa^{ns}	15.77 ± 0.24 Aa^{ns}	0.58 ± 0.04 Ab*	0.40 ± 0.01 Ab*	0.67 ± 0.03 Aa*
100 g Se ha^{-1} + I	9.07 ± 0.12 Aa^{ns}	1.50 ± 0.06 Ab*	15.57 ± 0.62 Aa^{ns}	0.72 ± 0.05 Aa^{ns}	0.42 ± 0.01 Ab*	0.58 ± 0.03 Bb*
200 g Se ha^{-1} + I	9.23 ± 0.10 Aa^{ns}	1.53 ± 0.07 Ab*	15.72 ± 0.61 Aa^{ns}	0.65 ± 0.05 Ab*	0.45 ± 0.02 Aa^{ns}	0.59 ± 0.02 Ab*
200 g Se ha^{-1} + I + Zn	8.94 ± 0.62	1.78 ± 0.09	16.32 ± 0.71	0.78 ± 0.06	0.46 ± 0.03	0.80 ± 0.03

N = nitrogen, P = phosphorus, K = potassium, Ca = calcium, Mg = magnesium, and S = sulfur. Uppercase letters compare the means with or without iodine within each Se rate and lowercase letters compare the means of Se rates at each iodine condition (with or without), according to Scott-Knott's test ($p \leq 0.05$). "ns" and "*" indicate non-significant and significant differences of the additional treatment (Se at 200 g ha^{-1} + I at 0.5 kg ha^{-1} + Zn at 2,5 kg ha^{-1}) compared with the others means by the Dunnett's test ($p \leq 0.05$).

Table 2. Mean contents of micronutrients (mg kg^{-1}) in the dry matter of sweet potato roots.

Treatment	Zn	B	Cu	Fe	Mn	Mo	Ni
				mg kg^{1}			
0 g Se ha^{-1}	5.56 ± 0.47 Aa*	3.11 ± 0.16 Aa^{ns}	4.42 ± 0.09 Aa*	14.14 ± 0.29 Ab^{ns}	4.32 ± 0.22 Ab*	0.29 ± 0.02 Bb^{ns}	0.13 ± 0.01 Ac*
50 g Se ha^{-1}	5.72 ± 0.17 Aa*	3.17 ± 0.04 Ba^{ns}	4.02 ± 0.11 Bb^{ns}	15.97 ± 1.03 Aa*	6.03 ± 0.30 Aa^{ns}	0.35 ± 0.03 Aa*	0.23 ± 0.01 Ab^{ns}
100 g Se ha^{-1}	5.32 ± 0.36 Aa*	3.33 ± 0.30 Aa^{ns}	3.78 ± 0.18 Bb^{ns}	13.78 ± 0.28 Bb^{ns}	6.06 ± 0.14 Ba^{ns}	0.30 ± 0.02 Aa*	0.15 ± 0.01 Bc*
200 g Se ha^{-1}	6.18 ± 0.48 Aa*	2.96 ± 0.13 Aa^{ns}	3.79 ± 0.12 Bb^{ns}	14.43 ± 0.71 Bb^{ns}	5.24 ± 0.20 Bb*	0.24 ± 0.02 Ab^{ns}	0.27 ± 0.01 Aa*
0 g Se ha^{-1} + I	5.27 ± 0.11 Aa*	3.42 ± 0.37 Ab^{ns}	4.34 ± 0.10 Aa*	12.63 ± 0.52 Ab^{ns}	4.76 ± 0.38 Ab*	0.34 ± 0.01 Aa*	0.10 ± 0.01 Bc*
50 g Se ha^{-1} + I	6.46 ± 0.56 Aa*	4.89 ± 0.43 Aa*	4.53 ± 0.14 Aa*	14.90 ± 0.77 Bb^{ns}	5.20 ± 0.16 Bb*	0.32 ± 0.01 Aa*	0.08 ± 0.01 Bc*
100 g Se ha^{-1} + I	5.68 ± 0.33 Aa*	3.64 ± 0.23 Ab^{ns}	4.29 ± 0.19 Aa*	15.96 ± 1.20 Aa*	7.83 ± 0.32 Aa*	0.25 ± 0.02 Bb^{ns}	0.20 ± 0.02 Ab^{ns}
200 g Se ha^{-1} + I	5.36 ± 0.48 Aa*	3.29 ± 0.29 Ab^{ns}	4.63 ± 0.30 Aa*	16.35 ± 0.69 Aa*	8.33 ± 0.37 Aa*	0.27 ± 0.00 Ab^{ns}	0.24 ± 0.02 Aa*
200 g Se ha^{-1} + I + Zn	7.80 ± 0.63	3.27 ± 0.30	4.01 ± 0.22	14.51 ± 0.85	5.61 ± 0.49	0.23 ± 0.02	0.21 ± 0.01

Zn = zinc, B = boron, Cu = copper, Fe = iron, Mn = magnesium, Mo = molybdenum, and Ni = nickel. Uppercase letters compare the means with or without iodine within each Se rate and lowercase letters compare the means of Se rates at each iodine condition (with or without), according to Scott-Knott's test ($p \leq 0.05$). "ns" and "*" indicate non-significant and significant differences of the additional treatment (Se at 200 g ha^{-1} + I at 0.5 kg ha^{-1} + Zn at 2,5 kg ha^{-1}) compared with the others means by the Dunnett's test ($p \leq 0.05$).

3.7 Selenium or iodine amounts remaining in the soil after harvesting

Considering the production of sweet potato biomass at the end of the crop cycle and based on Se/I contents found on it, we calculated the Se/I amount, in g ha^{-1}, that was left in the soil after harvesting, as can be seen in Table 3. As expected, Se amounts that were that kept in the soil after harvesting increased upon increasing the Se rates applied, irrespectively of I addition. The greatest Se amount that kept in the soil was verified for the additional treatment, i.e., when the highest Se rate (200 g ha^{-1}) was applied with I (0.5 kg ha^{-1}) and Zn (2.5 kg ha^{-1}), accounting for 43 g ha^{-1} of Se. On the other hand, I amounts that remained in the soil after harvesting were much lower, with the highest values being verified when I was co-applied with Se at 50 and 100 g ha^{-1}.

Table 3. Estimated Se or I amounts (g ha^{-1}) that remained in the soil after harvesting.

Se rates	Se content (mg kg^{-1} DM)	I content (mg kg^{-1} DM)	Shoot (kg ha^{-1} DM)	Se residual (g ha^{-1})	I residual (g ha^{-1})
0 g Se ha^{-1}	0.000	-	8,660	0.0000 Ad*	-
50 g Se ha^{-1}	1.065	-	9,738	10.371 Ac*	-
100 g Se ha^{-1}	1.951	-	9,471	18.478 Ab*	-
200 g Se ha^{-1}	3.020	-	9,938	30.013 Aa*	-
0 g Se ha^{-1} + I	0.000	0.217	8,555	0.000 Ad*	1.773 b^{ns}
50 g Se ha^{-1} + I	0.897	0.348	9,191	8.244 Ac*	3.053 a*
100 g Se ha^{-1} + I	1.739	0.359	9,427	16.393 Ab*	3.216 a*
200 g Se ha^{-1} + I	3.331	0.238	9,237	32.437 Aa*	2.084 b^{ns}
200 g Se ha^{-1} + I + Zn	4,500	0.258	9,754	43.893	2.409

DM = dry matter. Uppercase letters compare the means with or without iodine within each Se rate and lowercase letters compare the means of Se/I rates at each iodine condition (with or without), according to Scott-Knott's test ($p \leq 0.05$). "ns" and "*" indicate non-significant and significant differences of the additional treatment (Se at 200 g ha^{-1} + I at 0.5 kg ha^{-1} + Zn at 2,5 kg ha^{-1}) compared with the others means by the Dunnett's test ($p \leq 0.05$).

4 DISCUSSION

Crops respond differently to nutrient application and results of this present study clearly showed that agronomic biofortification by applying increasing foliar Se rates, alone or in combination with I and Zn, was effective in enhancing shoot and root Se contents in sweet potato plants without, in general, compromising crop yield (Fig. 1a, b). These findings are in accordance with the study conducted by Song et al. (2018), who evaluated foliar Se application on sweet potatoes in an experimental area in China.

The authors reported that sweet potato responded positively to the application of 30 and 60 g Se ha^{-1}, accumulating 0.31 mg Se kg^{-1} of dry sweet potato with this higher Se rate tested, a value of Se content 8.54 fold higher when compared with the control. Agreeing with the present study, Song et al. (2018) also verified that the application of Se did not significantly affect the production of root dry mass.

A study assessing the combined application of Se and I as foliar sprays in chicory was also carried out elsewhere (Germ et al., 2020), and the authors also reported that there were no significant effects of Se/I addition on plant biomass. Also, some other studies have found no effects of Se and I foliar application or the addition of both combined on the yield or biomass of buckwheat, pumpkins, and kohlrabi (Germ et al., 2019; Golob et al., 2020a; Golob et al., 2020b). Zou et al. (2019) observed that simultaneous foliar application of Zn, I, and Se as a cocktail significantly increased Zn, I, and Se contents in grains, without however changing grain yield. In addition to enriching crops with Se and I, it has to be stated that these elements play important roles in the plant's antioxidant system, protecting plants from different forms of oxidative stress, while also stimulating plant growth when there is stress (Golubkina et al., 2021; Hatfield et al., 2014; Kiferle et al., 2021; Gonzali et al., 2017).

Our study has demonstrated that Se contents determined in shoots and roots of sweet potato were practically not influenced by I addition (Fig. 3a, b), with I contents being influenced by Se rates, where higher I contents were verified when lower Se rates were applied (Fig. 6). Smolen et al. (2019), studying the interaction between Se x I applied via soil, reported that the application of 4 kg I ha^{-1} and 0.25 kg Se ha^{-1} allowed to elevate the accumulation of both elements in the storage roots of all tested cultivars of carrots. In another study, comparing the application of KI via soil and KIO_3 via foliar for potato biofortification, it was reported that both application methods were efficient for biofortifying potato plants with I (Smolen et al., 2020). In the study of Smolen et al. (2020), foliar I addition at 2 kg I ha^{-1} was more efficient in increasing I contents without changing starch and soluble sugar levels. The authors emphasized that the increment of I content was five times greater with foliar application compared with soil application, reaching 1.55 mg I kg^{-1}. In the present study, applying 0.5 kg I ha^{-1} of the same iodine source via foliar, the maximum I content found in sweet potato roots was 0.2 mg I kg^{-1}.

Selenium is known to be efficiently transported through the xylem, being highly mobile in the plant's phloem. Thus, foliar addition of Se at relatively low rate is suggested to achieve adequate Se levels in food crops for human nutrition (Lyons, 2018). By contrast, Zn concentrations in fruits, seeds, and tubers are severely limited by Zn transport in the phloem (White and Broadley, 2011). Although Zn is immobile within the plant, Zn translocation in the phloem after foliar application of Zn-based fertilizers has been found to be nutritionally significant for the growth and development of several plant species (Erenoglu et al., 2011; Mao et al., 2014). Once absorbed from the soil, I content decreases from the root to the leaf, stem, and fruit, with its transport also carried out mainly by the xylem (Hong et al., 2008; Li et al., 2016). Foliar spray studies have also shown that the I transport occurs mainly by the xylem rather than the phloem, suggesting that I accumulation in fruits, tubers, or seeds is low (Medrano-Macías et al., 2016; Lawson et al., 2015; Caffagni et al., 2012). Nevertheless, some fruit and tuber vegetables, such as carrots (Signore et al., 2018), potatoes (Smolen et al., 2020), strawberries (Li et al., 2016), and tomatoes (Kiferle et al., 2013) demonstrated the existence of a phloematic route, capable of providing I in their edible parts in contents that are relevant for biofortification, mainly occurring during the generative stage, transporting I to fruits, storage roots or tubers. The effectiveness of I transport from plant tissues to grains, fruits, and tubers is probably related to factors such as plant species, I concentration in the spray solution, and uniformity of leaf coverage during foliar application (Prom-u-thai et al., 2020).

Moreover, the biofortification efficacy with I depends on interaction with other elements. As found in this study, I contents in sweet potato plants were lower when Se was applied at higher rates (100 and, 200 g ha^{-1}, and 200 g ha^{-1} + Zn). Similar results were described by Zou et al. (2019), who reported that low I contents in wheat grains might be attributed to the inhibitory effect of other micronutrients in the cocktail (*i.e.*, Zn, Se or Fe), which may affect leaf absorption and/or translocation of I from wheat leaves to grains. On the other hand, positive effects of combining Se and I for increasing I contents in plants were achieved in other studies (Smolen et al., 2019; Golob et al., 2020a; Golubkina et al., 2020), similar to what we found here when Se was added at the lowest tested rate (50 g ha^{-1}).

Combined foliar application of Se, Zn, and I have shown to be relevant for increasing Se and Zn contents in sweet potato roots (Fig. 3b and Table 2). This fact is attributed to the effect of Zn and agrees with the study conducted by Mangueze et al. (2018), where the simultaneous foliar application of Se and Zn significantly increased Se and Zn contents in rice grains. In this study, we have found much higher Se contents in sweet potato when Zn was present in the applied solution. This increase is pronounced because Zn is an enzymatic cofactor that participates in the biosynthesis of nitrogen and compounds such as carboxylic acids, organic acids, and amino acids (Roda et al., 2020). More specifically, Zn is involved in protein synthesis, being a cofactor for many enzymes, including those related to the formation of peptide bonds, which are the bonds for joining amino acids to form proteins (Roda et al., 2020). After being absorbed by plants, Se is largely incorporated into amino acids (selenomethionine and selenocysteine), which form high-molecular proteins (Bañuelos, et al., 2022), and I also has been found covalently bound to proteins (Kiferle et al., 2021). Therefore, greater Zn contents in plants may favor protein formation and, as a result, allow increasing Se and I accumulation in plants (Persson, et al., 2016).

Selenium removal, *i.e.,* Se fraction found in edible parts compared with Se rates applied, was low, and much higher than I removal (Fig. 4). This is due to the greater accumulation of Se in other plant organs, such as leaves and stems in this experiment. Iodine accumulation in fruits and seeds is known to be possible but care should be taken to study the right application moment and placement to increase uptake efficiency (Medrano-Macías et al., 2016; Lawson et al., 2015; Caffagni et al., 2012). There are several factors affecting micronutrients mobilization and translocation within plants, such as type of micronutrient, plant phenological stage, environmental conditions, and the presence of other nutrients (White et al., 2004). Therefore, the foliar application provides readily available micronutrients to be taken up by plant tissue, prioritizing the nutrient accumulation in the final product (Cakmak and Kutman, 2018). In wheat plants, even when I was applied by foliar spray, I mobilization from leaves to grains was low (0.2–1.1%), being this mobilization cumulative, *i.e.* iodine moves from leaves to grain after each I application (Hurtevent et al., 2013).

In particular, the sources of foliar solutions applied in this field experiment considered the higher water solubility of selenate (SeO_4^{2-}) compared with selenite (SeO_3^{2-}) (Schiavon and Pilon-Smits, 2017) and the lower plant toxicity of iodate (IO_3^-) compared with iodide (I^-) (Cakmak et al., 2017). To facilitate the transport of the solution across the cell membrane, during the application of the treatments, 2% of KNO_3 and surfactant were added. In this context, Cakmak et al. (2017) reported that the addition of these additives into the applied solution significantly increased I contents in grains, compared with I contents verified when the applied solution did not contain additives.

This study showed that biofortifying sweet potatoes via the foliar application of Se and I may be a strategy for increasing Se/I contents in edible parts and consequently enhancing Se and I intake by population. For example, the application of 50 g Se ha^{-1} + I (0.5 kg ha^{-1}) could provide approximately 19.1 and 7.7 µg person^{-1} day^{-1} of Se and I respectively (Fig. 7a). These possible intakes of Se and I represent approximately 27.3% and 5.15% of the daily requirements of Se and I, respectively. Although higher Se intake may be found applying greater Se rates, this contribution of 27.3% is relevant, since people need to ingest different types of foods. Moreover, this lower Se rates (50 g ha^{-1}) increased I accumulation and its application may be more cost effective than higher Se rates. The values of Se, I and Zn intake presented in this study were estimated taking into account the consumption by sweet potato consumers of 156 g of sweet potato (FW) per day (Horodyska et al., 2021).

Considering that the consumption of sweet potatoes should be associated with the consumption of other foods rich in Se (Ross et al., 2012), Se intake values reported in this present work show great potential to help prevent Se deficiency in humans exposed to a Se-poor diet. However, further studies to evaluate Se and I bioaccessibility in sweet potatoes enriched through agronomic biofortification by co-application of those elements are suggested and required to better define the ideal rates for foliar application of Se/I in this crop. Also, assessment of the effects of storage and cooking also needs to be taking into account, since we estimated Se/I intake considering their contents determined in the pelled row sweet potato.

Finally, this study has presented Se/I amounts that remained in the soil after harvesting, *i.e.,* Se/I that was in the plant shoot (Fig. 1a). Considerable amounts of Se were still present in the area where sweet potato was grown (approximately from 10 to 30 g ha^{-1} depending on Se rates applied and approximately 40 g ha^{-1} when Zn was also applied together the higher Se rate). Lessa et al. (2020), studying rice biofortification by soil Se application, reported that rice grains could reach adequate levels of Se when approximately 40 g ha^{-1} of Se is applied in a tropical soil as sodium selenate applied via leaf during the reproductive phase of the plant. There are indications that Se can be recycled from crop residue, as shown by dos Santos et al. (2021) in Mombaça grass plants that increased Se content in their dry matter when grown in a clayey soil previously cultivated with common beans under different selenate application rates.

5 CONCLUSION

Sweet potato has shown to be an important crop for Se biofortification, with Se contents in edible parts being increased upon increasing Se rates applied via foliar, irrespectively of I addition. At a low level of Se applied in combination with I, the total sweet potato dry yield was improved. The addition of I at 0.5 kg ha^{-1} did not influence Se contents found in sweet potato, but Se rates affected I contents, where the highest Se rates reduced I contents. Thus, the higher I content was detected when combined with Se at 50 g ha^{-1}. The additional treatment, including Zn (2.5 kg ha^{-1}) in the solution applied in combination with Se (at 200 g ha^{-1}) and I (at 0.5 kg ha^{-1}) was relevant for increasing Se, I, and macronutrient contents in sweet potatoes. Based on sweet potato consumption, the biofortified sweet potato produced in this present study could contribute to a significant fraction of the recommended Se intake by humans (which is 70 µg day^{-1} person^{-1}). Selenium and I contents in sweet potato for the treatment when Se was applied at 50 g ha^{-1} in the presence of I were highlighted, although higher Se contents were detected with increasing Se rates applied. This treatment could provide 19.1 µg day^{-1} person^{-1} of Se (27.3 % of the RDA for Se), and 7.7 ug day^{-1} person^{-1} (5.15% of RDA for I). Therefore, future studies are suggested for more efficient methods of application of I to improve uptake efficiency, and assessing Se/I bioaccessibility in those biofortified sweet potato produced by the Se/I foliar application.

ACKNOWLEDGEMENT

The authors are grateful for the support of the Brazilian National Council for Scientific and Technological Development (CNPq-141057/2019-0 and 404412/2021-1). Also, we thank the Minas Gerais State Research Foundation (Fapemig-APQ-02812-18), and the Coordination for the Improvement of Higher Education Personnel (CAPES) for financial support. Also, we thank SQM for providing us with the KNO_3 used and for the financial support with I analysis. Finally, we thank the Faculty of Science at University of Nottingham for collaboration with Se/I analysis.

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ARTICLE 2: BIOFORTIFICATION OF SWEET POTATO WITH SELENIUM IN A TROPICAL SOIL: EFFECTS OF APPLIED UREA AND MAP

This article was prepared in line with the guidelines of the journal: Plants, which will be submitted to.

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Abstract

Due to the essentiality of selenium (Se) to humans and animals and the occurrence of Se deficiency in human nutrition, Se application in soils is one important alternative to increase Se contents in food crops. Selenium levels in plants are directly related to Se availability in the soil, and most Brazilian soils are Se deficient, with Se contents ranging from 0.01 to 0.2 mg Se kg^{-1} soil. This study aimed to assess under field conditions the biofortification of sweet potato plants with Se by applying Se-enriched fertilizer (urea and MAP) rates. Also, a Se adsorption test was carried out with soil samples collected at the site of the field experiments, with its desorption being evaluated by the addition of increasing rates of P_2O_5 and N (equivalents to 0, 100, 250, 500, and 1000 kg ha^{-1}) using both, pure salts or commercial fertilizers (MAP/urea). For biofortification, Se was applied at different rates (0, 50, 100, and 200 g ha^{-1}) using three different methods, as follows: Se-enriched monoammonium phosphate (Se-MAP), Se-enriched urea (Se-urea), and Se-enriched MAP + Se-enriched urea (Se-MAP+urea). Results indicate that Se contents in sweet potato shoots and roots increased upon increasing the Se-enriched fertilizer rates, in some cases positively affecting sweet potatoes yield. The highest Se contents in sweet potatoes were observed when 200 g ha^{-1} of Se-enriched urea was added to the soil. Based on the consumption of 156 g of fresh sweet potato roots, this treatment could contribute to providing 34.4 µg person^{-1} day^{-1} of Se, which is 49% of the recommended daily Se intake for an adult. Additionally, Se adsorption increased with increasing Se concentrations added in the solution, while Se desorption was greater following the addition of P (as a p.a. grade reagent) and N (urea).

Keywords: *Ipomoea batatas* L. Functional agriculture. Selenate. Monoammonium phosphate. Beneficial element. Tropical soil.

1 INTRODUCTION

Selenium (Se) is an essential element for humans and animals, playing important roles as an enzyme activator that regulates the antioxidant system and the production of thyroid hormones (Hatfield et al., 2014). Studies have shown that the proper intake of this element may positively affect the control and prevention of several human health problems, such as cardiovascular diseases, diabetes, cancer, inflammatory problems, infertility, and immune system disorders (Saha et al., 2017). According to estimates reported by Schiavon and Pilon-Smits (2017), one billion people are affected by Se deficiency worldwide. Although considered a beneficial element for plants, Se also plays a crucial role in plant metabolism, growth, and defense mechanisms (Feng et al., 2003).

The recommended daily intake of Se for adults is 70 µg day^{-1} for men and 60 and 75 µg day^{-1} for pregnant and lactating women, respectively (Kipp et al., 2015). Selenium contents found in crops are strongly dependent on the Se content in the soil and its availability (Lopes et al., 2017). In Se-poor areas, Se deficiency in humans and animals can be overcome through dietary diversification, supplementation, fortification by industry, or by the use of biofortification, a strategy that consists of increasing Se levels in the edible parts of food crops by introducing it into the fertilization (agronomic biofortification) or by genetic improvement (Schiavon and Pilon-Smith, 2017).

Soil is the main source of Se, with its greatest reserves being found in areas with volcanic activity or sedimentary rocks. In contrast, most arable land around the world has total Se levels ranging from 0.1 to 2.0 mg kg^{-1}, with an average of 0.4 mg kg^{-1} (Saha et al., 2017). Tropical soils, such as Brazilian soils of the Cerrado biome, are naturally poor in Se (0.01 to 0.2 mg Se kg^{-1}), highly weathered, and predominant with of 1:1 clays and Fe and Al oxides (Gabos et al., 2014; Silva Júnior et al., 2017; Mirlean et al., 2018; Carvalho et al., 2018).

Several studies have been conducted addressing the sorption behavior of Se in Brazilian soils (Mouta et al., 2008; Abreu et al., 2011; Gabos et al., 2014; Lessa et al., 2016; Araujo et al., 2018, 2020). Most studies have shown that selenite (Se IV) tends to be adsorbed mainly by an inner-sphere complex, while selenate (VI) forms an outer-sphere complex, the latter being more available (McBride, 1994; Araujo et al., 2020; Schiavon et al., 2020). Positive relationships between adsorption of Se, clay, and total Fe and Al oxides were found by Abreu et al. (2011), in a adsorption study conducted on soils of the Cerrado biome. Soils in this region of Brazil have higher amounts of oxides, which are known for their high capacity to adsorb anions (Pinto et al., 2013; Silva Júnior et al., 2017; Carvalho et al., 2018).

Selenium-enriched fertilizer has been proposed as a strategy for biofortification, *i.e.,* to increase the daily Se intake for animals and humans (Wu et al., 2015). Few countries have well-documented regulations for using Se-enriched fertilizers in crops. Finland is one of these examples (Alfthan et al., 2015), but this country has soils with distinct characteristics compared with those found in tropical environments.

Sweet potato (*Ipomoea batatas* L.) is a tuberous root widely used in human and animal nutrition. Its production and consumption occur mainly in developing countries due to its economic and social importance. Easily propagated and palatable, this vegetable spreads throughout the world due to its rusticity, low production cost, lower demand for agricultural inputs, and benefits that include: excellent source of energy, carbohydrates, fibers, nutrients, vitamins, bioactive compounds, anthocyanins, and phenolics compounds (Ramos et al., 2021; Frond et al., 2019). Little is known about the potential of this crop to accumulate Se in its tuberous roots, thus, studies evaluating the biofortification of sweet potatoes with Se are needed and of great relevance.

Additionally, there are few studies evaluate the stability of Se in tropical soils with the addition of phosphorus (P) and nitrogen (N) fertilizers, which is relevant for agronomic biofortification studies that use soil addition of Se as the preferred strategy. Therefore, this present work aimed to: (1) assess Se adsorption in the soil of the experimental area used for sweet potato cultivation; (2) evaluate the effect of Se-enriched urea and MAP on the agronomic biofortification of sweet potato, its growth, and chemical composition; (3) assess the influence of adding sources of P and N on Se desorption, in order to combine this with Se accumulation in the plant.

2 MATERIAL AND METHODS

2.1 Selenium adsorption

To conduct adsorption and desorption experiments, samples from 0-20 cm were collected from the experimental area before the installation of the experiment to carry out adsorption and desorption tests. Soils were air-dried, gently ground in an agate mortar to pass through a 2 mm sieve, and subjected to Se adsorption and desorption. For the adsorption experiment, 3.0 g of soil were weighed in triplicate, and suspended in 30 mL of a solution using 50 mL centrifuge tubes containing the following doses of Se, in the form of Na_2SeO_4 (mg L^{-1}): 0, 0.25, 0.5, 0.75, 1.0, 1.5, 3.0, 6.0, 12.0, 24.0, and 48.0.

The reaction solution was prepared in a background electrolyte solution of 15 mmol L^{-1} of sodium chloride (NaCl) and 0.1 M sodium acetate. The pH of the solution was adjusted to 5.0 ± 0.1. The reaction was carried out for seven days at constant stirring at 120 rpm in a horizontal shaker at room temperature. Then, tubes were centrifuged at 9250 rpm for 15 minutes at 22°C. The supernatant was collected and filtered through 0.22 µm membrane filters for subsequent Se analysis to calculate the amount of Se was adsorbed in each treatment. Selenium analyzes were performed using inductively coupled plasma optical emission spectrometry (ICP-OES). After the analyses, the amount of Se adsorbed (mg kg^{-1}) in the soil was calculated according to the equation 1:

$$Se_{ads} = ((C_i - C_e) * V) / M \qquad \text{Eq 1}$$

where: Se_{ads} (mg kg^{-1}) is the amount of Se adsorbed in the soil; C_i (mg L^{-1}) is the initial Se concentration in the solution; C_e (mg L^{-1}) is the equilibrium Se concentration after the adsorption reaction; V (L) is the final volume of the solution and M (kg) is the mass of the soil.

Se adsorption data were fitted to the Langmuir isotherm (2) using firstly its linearized form (3).

$q = (K_L * C_e * b_{max}) / (1 + (K_L * C_e)$ Eq 2

$C_e/q = 1 / (K_L * b_{max}) + (C_e * b_{max})$ Eq 3

where: q is the amount of Se adsorbed (mg kg^{-1}); C_e is the equilibrium Se concentration after the adsorption reaction (mg L^{-1}); b_{max} is the maximum adsorption capacity of Se estimated by the Langmuir model (mg kg^{-1}), and K_L is a constant of the Langmuir isotherm.

2.2 Selenium desorption by nitrogen and phosphorus

A desorption experiment was performed on soils that received 48 mg Se L^{-1} during the adsorption experiment because they had higher Se adsorption. For this, 30 mL of 15 mmol L^{-1} background electrolyte solution of NaCl with 0.1 M pH 5 sodim acetate was added to the soil remaining in the tube after centrifugation and decantation. Five different concentrations of nitrogen (N) and phosphorus (P) using both, pure reagents and commercial fertilizers were tested as desorptive solutions. In the case of N, solutions of ammonium nitrate (NH_4NO_3, Fisher Scientific, Waltham, MA, USA, ≥ 98.0% purity) and commercial urea [$CO(NH_2)_2$] were prepared with the following concentrations of N: 0, 2.30, 5.75, 11.5, and 23.0 mg L^{-1}. Phosphorus was added in the form of sodium phosphate (Na_2HPO_4, Fisher Scientific, Waltham, MA, USA, ≥ 99.0% purity) and commercial MAP ($NH_4H_2PO_4$) at the following rates of P: 0, 2.18, 5.46, 10.90, and 21.85 mg L^{-1}. The desorption reaction was carried out for seven days under constant stirring and after the reaction period, they were centrifuged, filtered, and analyzed by ICP-OES as mentioned before. After the analyses, Se desorbed amounts (mg kg^{-1}) were calculated by deducting the Se concentration remaining in the solution at equilibrium. The doses of N and P added for performing Se desorption were equivalent to applications of 0, 100, 250, 500, and 1000 kg ha^{-1} of N and P_2O_5

2.3 Field experiment and experimental design

The field experiment was conducted with an early sweet potato cultivar (Ligeirinha). The experiment was carried out under field conditions at 3W Agronegócios farm located in Carrancas, Minas Gerais State, Brazil (21°32′52” S; 44°43′24” W). The soil is classified as a typic dystrophic (Tb) Haplic Inceptisol

(CXbd), the average annual precipitation in the cultivated field was 1636 mm, and the average annual temperature was 18.7°C.

The experiment was established in a randomized block design, composed of ten treatments with 5 replicates, totaling 50 experimental plots with 18 m^2. Each plot consisted of 5 m long by 3.6 m wide (sweet potato row-spacing at 1.2 m), with the two central sweet potato lines (excluding 1 m in each extremity) being used as useful area for evaluating the variables measured in this study. Planting was made with four sweet potato stem-segments (0.25 m to 0.3 m) per linear meter and fertilization was carried out by applying 150 kg ha^{-1} of monoammonium phosphate (MAP = 10.5% N and 52% P_2O_5). Twenty-five days after sowing, additional nitrogen (N) and potassium (K) were top-dressed at the rate of 250 kg ha^{-1} of urea and 330 kg ha^{-1} of potassium chloride (KCl), respectively.

Selenium was applied as sodium selenate (Na_2SeO_4, Sigma-Aldrich, Saint Louis, MO, USA), p.a. ($\geq$ 98.0% purity) added at 50, 100, and 200 g ha^{-1} using three different methods, as follows: Se-enriched monoammonium phosphate (Se-MAP), Se-enriched urea (Se-urea), and Se-enriched monoammonium phosphate + Se-enriched urea (Se-MAP+urea). Based on the recommended fertilization rates, 18g of MAP and 30g of urea were applied per linear meter.

Physical and chemical characteristics of the soil in the experimental area before treatment applications were determined according to the methodology proposed by the Brazilian Agricultural Research Corporation (EMBRAPA) (Teixeira et al., 2017), and the soil characteristics were as follows: pH (H_2O) = 6.55; pH ($CaCl_2$) = 5.95; soil organic matter = 39.5 g kg^{-1}; P (Mehlich-1) = 1.15 mg dm^{-3}; P-rem = 24.6 mg L^{-1}; P-resin = 26.9 mg dm^{-3}; K = 245.50 mg dm^{-3}; S = 13.9 mg dm^{-3}; Ca = 3.90 cmolc kg^{-1}; Mg = 1.60 cmolc kg^{-1}; B = 0.44 mg dm^{-3}; Cu = 0.55 mg dm^{-3}; Fe = 31.9 mg dm^{-3}; Mn = 10.9 mg dm^{-3}; Zn = 0.95 mg dm^{-3}; Al = 0.10 cmolc kg^{-1}; H+Al = 1.95 cmolc kg^{-1}; SB = 6.15 cmolc kg^{-1}; CEC = 8.10 cmolc kg^{-1}; clay = 220 g kg^{-1}; silt = 110 g kg^{-1}; and sand = 670 g kg^{-1}.

2.4 Preparation and determination of selenium in Se-enriched urea and MAP

Considering the recommended amounts of urea and MAP, Se-enriched urea and MAP granules were prepared at the following concentrations (~ 333, 666, and 1,333 mg Se kg^{-1} for MAP) and (200, 400, and 800 mg Se kg^{-1} for urea) to reach the tested Se rates (50, 100 and 200 g ha^{-1}). The calculated amounts of Se were mixed with 1.0 kg of fertilizer until homogenization. To evaluate the homogeneity obtained during the addition of Se to the granules, 1.15 mL of the additive diethanolamine (adhesive) and 12 drops of red organic dye were added to the mixture aforementioned. Then, the final product of this mixture was spread over a bench for drying and stored in a place away from humidity due to the hygroscopic characteristics of fertilizers.

For determining Se contents in the final Se-enriched fertilizer as well as in the commercial urea and MAP, random sampling of each fertilizer was carried out. A sample composed of approximately 5 g of each fertilizer was ground and sieved through a 100-mesh nylon sieve (< 150 µm) to determine Se content using the 3051A digestion method described by the United States Environmental Protection Agency – USEPA (USEPA, 2007). In this digestion, approximately 0.5 g of the ground and sieved fertilizers were weighed in triplicate, which were subsequently digested with 5 mL of conc. $HNO_3 \geq 65\%$. The extracts were allowed to stand overnight at room temperature and digestion was carried out the next day. For this purpose, Teflon tubes were sealed and placed in a microwave oven (Mars 5CEM Corporation, Matthews, EUA), with a temperature set at 175°C and a controlled pressure of 0.76 MPa for 15 minutes. After digestion, the extracts were cooled to room temperature and filtered through a paper filter. Then, 5 mL of deionized water was added to the final volume. After the digestion, Se was analyzed by atomic absorption spectroscopy with electro-thermal atomization in a graphite furnace (Perkin Elmer, model AA-analyst 800, Midland, Canada). The average recovery of Se in Se-enriched fertilizers was 96.3% (n = 3).

2.5 Determination of selenium, macronutrient, and micronutrient contents in biofortified sweet potato

Sweet potato plants were grown for 159 days. During the harvesting, shoots of all plots were collected and their fresh weight was recorded. Then, a sample of the shoot from each experimental plot was transported to the laboratory, where they were dried in an oven with forced air circulation at 65°C for 72 h to reach a constant weight. Then, the dry weight of the shoots was also recorded. The harvested potato (root) was first classified as commercial sweet potatoes according to Azevedo et al. (2002) and Huáman (1992) and those that did not fit as commercial were discarded. Similarly, fresh sweet potato was also weighed and then dried in an oven with forced air circulation at 65°C for 72 h, being weighed again for recording the dry matter (DM) of potato roots. After that, both materials (shoot and root/sweet potato) were ground in a 1.0 mm Willey mill for further analysis.

Selenium and other elements contents in sweet potato were determined in the extracts following acid digestion of plant material using concentrated HNO_3 in a microwave oven (Mars 5CEM Corporation, Matthews, EUA), according to the 3051A method (USEPA, 2007). The extracts were analyzed by atomic absorption spectroscopy with electro-thermal atomization in a graphite furnace (Perkin Elmer, model AA-analyst 800, Midland, Canada) for determining Se, and by inductive coupled plasma emission spectrometry (ICP-OES) for determining the other elements. Total N contents were also determined by the Kjeldahl method. For quality assurance and control, a sample of the standard reference material (Tomato Leaves - NIST, National Institute of Standards and Technology, Gaithersburg, USA) with a known content of Se (6.7 mg Se kg^{-1}) was included in each wet-acid digestion batch. The mean recovery for Se in this reference material was 93.4%.

Considering the Se contents determined in the shoots (g of Se kg^{-1} DW) and the total production of dry matter of shoot, the amount of Se from shoots, in g ha^{-1}, that would remain in the soil after sweet potato harvesting, termed here as residual Se, was estimated using the equation (4):

$$Se_{residual}\ (g\ ha^{-1}) = [(Se\ in\ DM\ (mg\ kg^{-1}) * DM\ of\ shoot\ (kg\ ha^{-1})] / 1000 \qquad \text{Eq 4}$$

Moreover, the fraction of the applied Se that was incorporated in sweet potato roots and then removed (Se removal) was calculated as follows (equation 5):

$$Se_{removal}\ (\%) = (((Se_{treatment} - Se_{control}) * Y) / Se_{rate}) * 100 \qquad \text{Eq 5}$$

where: $Se_{removal}$ (%) is the use efficiency of Se rates applied in the leaves by harvested sweet potato roots; $Se_{treatment}$ (g kg^{-1}) = Se contents in sweet potato from plants grown in treatments that received Se application; $Se_{control}$ (g kg^{-1}) = Se contents in sweet potato roots from plants grown in treatments without Se applications; Y = Yield of sweet potato, in dry weight (kg ha^{-1}); Se_{rate} (g ha^{-1}) = Se rates applied in the treatment.

According to Horodyska et al. (2021), the average daily consumption of sweet potatoes by consumers in Brazil is 156 g $person^{-1}$ day^{-1}. Based on this information and taking into account the Se contents determined on the dried powdered peeled sweet potato of the present study, we have also calculated the possible Se intake from the consumption of these biofortified roots, using equation (6) below:

$$Se_{intake} = Se_{treatment} * DM * C \qquad \text{Eq 6}$$

where: Se_{intake} (μg person^{-1} day^{-1}) is the daily Se intake estimation per person, $Se_{treatment}$ (μg kg^{-1}) is the Se contents in peeled sweet potato found for the studied treatments that received Se application, DM (%) is the percentage of dry matter of the harvested sweet potato (25%), and C (kg person^{-1} day^{-1}) is the mean consumption of fresh sweet potato per person from Brazilian consumers (Horodyska et al., 2021).

2.6 Statistical analysis and data processing

Data were submitted to analysis of variance and treatment means were compared by Scott Knott's test ($p \leq 0.05$) using the Sisvar software, version 5.6 (Ferreira, 2011). The control treatment (Se rate = 0) was compared by Dunnett's test ($p \leq 0.05$) against all treatments and included in the graphs through dashed lines. Graphics were performed using the Sigma Plot software, version 12.0 (Systat Software Chicago, IL, USA).

3 RESULTS

3.1 Se adsorption

The linearized Langmuir isotherm is presented in Figure 1a. The maximum Se adsorption capacity (b_{max}) of the assessed soil, estimated by the Langmuir model, was 45.87 mg kg^{-1}. Selenium adsorption increased with increasing Se equilibrium concentration in solution (Fig. 1b), following the Langmuir model and almost reached a constant plateau in the highest Se concentration added. The amount of adsorbed Se(VI) at highest equilibrium concentration (*i.e.,* 48 mg kg^{-1} Se) was 24.32 mg Se kg^{-1}.

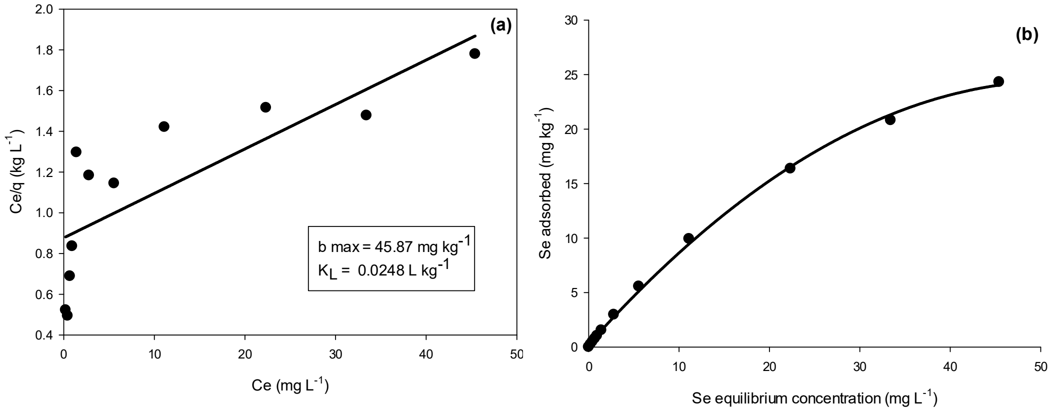

Fig. 1. Linearized Langmuir isotherm (a) and selenium adsorbed (mg kg^{-1}) as a function of Se equilibrium concentration (mg L^{-1}) in solution (b). Points indicate experimental data, while lines were obtained using the Langmuir model.

3.2 Selenium desorption by phosphorus and nitrogen

Desorption results in response to P and N addition revealed that Se desorption from the soil increased with increasing P and N concentrations (Fig. 2), depending on their source, *i.e.,* whether they are a p.a. grade reagent or fertilizers. Significant linear increases in Se desorption were found by adding P as a pure reagent - sodium phosphate (Fig. 2a) and by adding N as urea (Fig. 2b). When these nutrients were added as MAP and N as pure salt (ammonium nitrate), the increases observed on Se desorption were not significant.

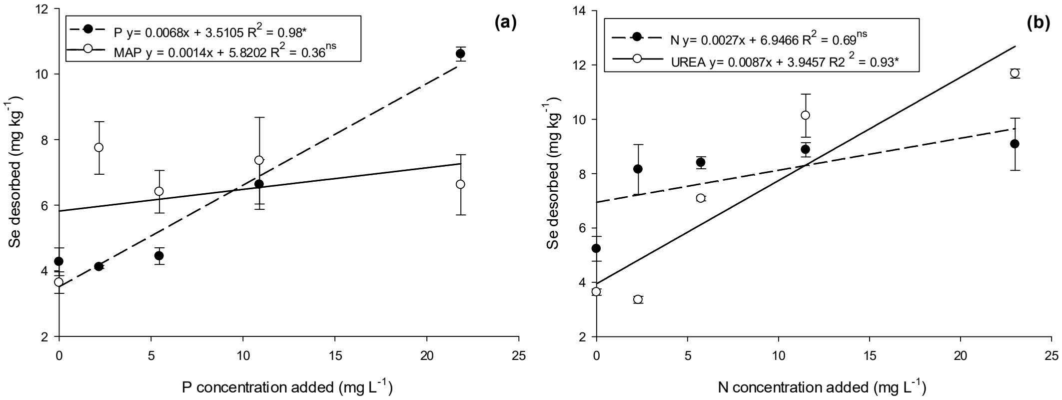

Fig. 2. Selenate desorbed as a function of P (a) and N (b) concentrations added in solution: “ns” and "*" indicate non-significant and significant differences of regressions ($p \leq 0.05$).

3.3 Production of shoot and sweet potato yield

Production of shoot dry matter (SDM) and root dry matter (RDM) (Fig. 3) were influenced ($p \leq 0.05$) by the interaction between the studied factors (Se-enriched fertilizers and Se rates). There were no significant effects of the Se source/enriched fertilizers on SDM within each Se rate (Fig. 3a). Among the applied fertilizers, the increase of Se rates also did not influence the SDM, except for the treatment that received Se-enriched MAP at 200 g ha^{-1}, where higher SDM were verified. The lowest SDM values were observed in the control treatment and 50 g Se ha^{-1} via urea. Contrariwise, the production of RDM varied considerably among Se rates and Se-enriched fertilizers (Fig. 3b), with the highest values being 9.20 and 8.72 t ha^{-1}, which were found when 200 and 100 g ha^{-1} of Se was applied with urea and urea + MAP, respectively.

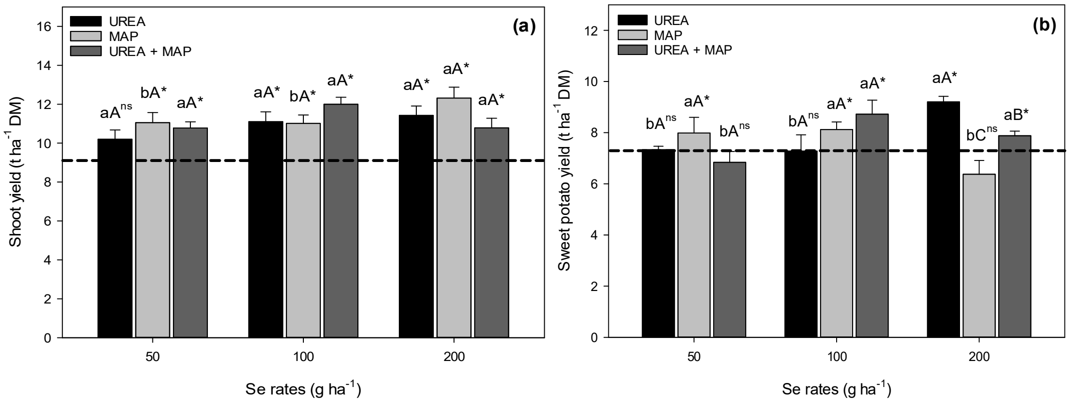

Fig. 3. Production of shoot dry matter (a) and sweet potato/root dry matter (b). Uppercase letters compare the means of Se-enriched fertilizers within each Se rate and lowercase letters compare the means of Se rates at each Se-enriched fertilizer condition (urea, MAP, or urea + MAP), according to Scott-Knott's test ($p \leq 0.05$). The dashed line refers to the value found for the control treatment. "ns" and "*" indicate non-significant and significant differences, respectively, of the control treatment compared with the other bars by the Dunnett's test ($p \leq 0.05$). Vertical bars indicate the standard error of average values (n = 5).

3.4 Selenium contents in shoot and root

Selenium content in sweet potato shoots and roots increased upon increasing Se rates (Fig. 4) with the highest Se content being observed when Se-enriched fertilizers were applied at 200 g ha^{-1}. The application of the different Se-enriched fertilizers had a significant effect on Se content in shoots at rates of 50 and 100 g Se ha^{-1} (Fig. 4a). Also, Se contents found in sweet potato roots changed depending on Se-enriched fertilizers (sources) when Se was added at 100 and 200 g ha^{-1}. In both Se rates (100 and 200g ha^{-1}), higher Se contents were found with Se-enriched urea, being 0.44 and 0.88 mg kg^{-1}, respectively (Fig. 4b). The lowest Se contents in sweet potato shoots and roots were observed in the control treatment due to the absence of Se application in this treatment.

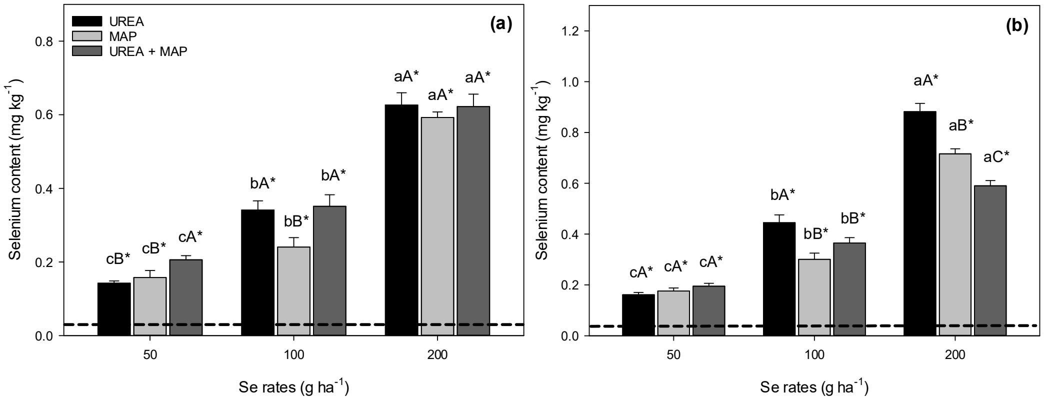

Fig. 4. Selenium contents in sweet potato shoots (a) and roots (b) after harvesting. Uppercase letters compare the means of Se-enriched fertilizers within each Se rate and lowercase letters compare the means of Se rates at each Se-enriched fertilizer condition (urea, MAP, or urea + MAP), according to Scott-Knott's test ($p \leq 0.05$). The dashed line refers to the value found for the control treatment. "ns" and "*" indicate non-significant and significant differences, respectively, of the control treatment compared with the other bars by the Dunnett's test ($p \leq 0.05$). Vertical bars indicate the standard error of average values (n = 5).

3.5 Estimation of daily intake of Se by consuming biofortified sweet potato and Se removed

The recommended daily allowance (RDA) of Se for adults is 70 µg person^{-1} day^{-1} (Kipp et al., 2015). Considering the average consumption of fresh sweet potato consumed by Brazilian consumers of 156 g person^{-1} day^{-1} (Horodyska et al., 2021), the estimation of Se intake by consuming the biofortified sweet potato produced in this present study was calculated and presented in Figure 5a. Potential daily Se intake increased upon increasing Se rates applied as Se-enriched fertilizers. Similar to what was reported for Se content in root, there was a significant effect of Se-enriched fertilizers within each rate. As highlighted in the results of Se contents, the application of 200 g ha^{-1} of Se-enriched urea resulted in higher Se contents in sweet potato root. As a consequence, the higher Se intake estimation was obtained in this treatment, accounting for 34.4 µg person^{-1} day^{-1} (49% of the RDA).

Selenium removal by sweet potato roots showed significant differences between Se rates and Se-enriched fertilizers. The percentage of Se removed ranged from 2.23% to 3.98% (Fig. 5b), reaching the highest removal at 200 g ha^{-1} with Se-enriched urea. Selenium removal found with Se-enriched urea at 200 g ha^{-1} was much higher than those observed when this same Se rate was applied as Se-enriched MAP or with MAP+urea.

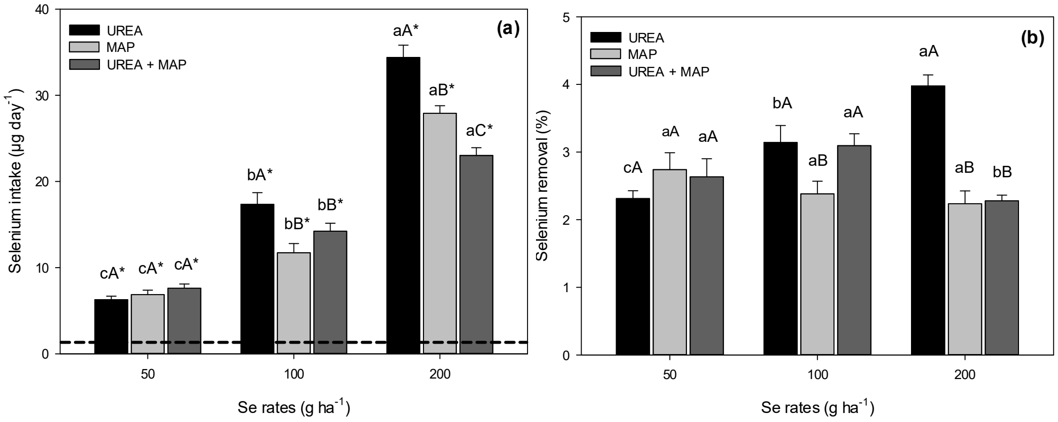

Fig. 5. Estimation of potential daily Se intake (a) per person by consuming the biofortified sweet potato produced in this present study (dry matter of sweet potato = 75%), and Se removal (%) in sweet potato roots (b) after harvesting. Uppercase letters compare the means of Se-enriched fertilizers within each Se rate and lowercase letters compare the means of Se rates at each Se-enriched fertilizer condition (urea, MAP, or urea + MAP), according to Scott-Knott's test ($p \leq 0.05$). The dashed line refers to the value found for the control treatment. "ns" and "*" indicate non-significant and significant differences, respectively, of the control treatment compared with the other bars by the Dunnett's test ($p \leq 0.05$). Vertical bars indicate the standard error of average values (n = 5).

3.6 Macro and micronutrients in sweet potato root

The chemical composition of macronutrients in the dry matter of sweet potato roots demonstrated that there was no significant effect of Se rates and Se-enriched fertilizers on phosphorus (P), potassium (K), magnesium (Mg), and sulfur (S) contents (Table 1). The highest nitrogen (N) contents were observed for control treatment, and applying 50 and 200 g ha^{-1} Se-enriched urea. No trend was found for the measured calcium (Ca) contents.

Similar to what has been described for most macronutrients, boron (B), copper (Cu), iron (Fe), magnesium (Mg), and zinc (Zn) showed no significant differences among Se rates and Se-enriched fertilizers, except for Fe content on Se-enriched MAP, where higher the content was observed at 50 g Se ha^{-1} (Table 2).

Table 1. Average chemical composition of macronutrients (g kg^{-1}) in the dry matter of sweet potato roots

Treatments	N	P	K	Ca	Mg	S
	g kg^{-1}					
Control	8.93 ± 0.14	1.35 ± 0.04	16.60 ± 0.62	0.45 ± 0.02	0.51 ± 0.03	0.66 ± 0.02
50 g Se ha^{-1} - UREA	8.96 ± 0.21Aa^{ns}	1.29 ± 0.05Aa^{ns}	14.85 ± 0.43Aa^{ns}	0.33 ± 0.01Aa*	0.44 ± 0.03Aa^{ns}	0.57 ± 0.02Aa^{ns}
100 g Se ha^{-1} - UREA	8.25 ± 0.43Aa*	1.22 ± 0.05Aa^{ns}	14.67 ± 0.65Aa^{ns}	0.33 ± 0.02Ba*	0.42 ± 0.03Aa^{ns}	0.59 ± 0.03Aa^{ns}
200 g Se ha^{-1} - UREA	8.72 ± 0.28Aa^{ns}	1.31 ± 0.03Aa^{ns}	14.61 ± 0.68Aa^{ns}	0.39 ± 0.03Aa*	0.43 ± 0.02Aa^{ns}	0.59 ± 0.01Aa^{ns}
50 g Se ha^{-1} - MAP	8.10 ± 0.09Ba*	1.19 ± 0.07Aa^{ns}	15.37 ± 0.65Aa^{ns}	0.37 ± 0.02Aa^{ns}	0.43 ± 0.02Aa^{ns}	0.57 ± 0.04Aa^{ns}
100 g Se ha^{-1} - MAP	8.35 ± 0.48Aa*	1.28 ± 0.10Aa^{ns}	14.33 ± 0.63Aa^{ns}	0.34 ± 0.01Ba*	0.40 ± 0.03Aa^{ns}	0.60 ± 0.05Aa^{ns}
200 g Se ha^{-1} - MAP	7.93 ± 0.31Aa*	1.18 ± 0.05Aa^{ns}	12.96 ± 0.64Aa^{ns}	0.35 ± 0.01Aa*	0.37 ± 0.02Aa^{ns}	0.51 ± 0.02Ba^{ns}
50 g Se ha^{-1} - UREA + MAP	7.73 ± 0.25Ca*	1.23 ± 0.09Aa^{ns}	14.70 ± 0.59Aa^{ns}	0.35 ± 0.03Aa*	0.41 ± 0.01Aa^{ns}	0.64 ± 0.02Aa^{ns}
100 g Se ha^{-1} - UREA + MAP	7.77 ± 0.50Aa*	1.06 ± 0.10Aa^{ns}	13.63 ± 0.56Aa^{ns}	0.41 ± 0.02Aa^{ns}	0.39 ± 0.01Aa^{ns}	0.54 ± 0.04Aa^{ns}
200 g Se ha^{-1} - UREA + MAP	8.00 ± 0.25Aa*	1.13 ± 0.03Aa^{ns}	13.03 ± 0.46Aa^{ns}	0.29 ± 0.02Aa*	0.39 ± 0.01Aa^{ns}	0.54 ± 0.01Ba^{ns}

N = nitrogen, P = phosphorus, K = potassium, Ca = calcium, Mg = magnesium, and S = sulfur. Uppercase letters compare the means of Se-enriched fertilizers within each Se rate and lowercase letters compare the means of Se rates at each Se-enriched fertilizer condition (urea, MAP, or urea + MAP), according to Scott-Knott's test ($p \leq 0.05$). "ns" and "*" indicate non-significant and significant differences, respectively, of the control treatment compared with the other bars by the Dunnett's test ($p \leq 0.05$).

Table 2. Average chemical composition of micronutrients (mg kg^{-1}) in the dry matter of sweet potato roots.

Treatments	B	Cu	Fe	Mn	Zn
			mg kg^{-1}		
Control	3.41 ± 0.45	5.88 ± 0.35	17.32 ± 1.42	4.27 ± 0.20	6.31 ± 0.22
50 g Se ha^{-1} - UREA	2.60 ± 0.26Aa^{ns}	5.32 ± 0.19Aa^{ns}	20.46 ± 2.67Aa^{ns}	4.21 ± 0.38Aa^{ns}	6.00 ± 0.51Aa^{ns}
100 g Se ha^{-1} - UREA	2.94 ± 0.54Aa^{ns}	5.91 ± 0.45Aa^{ns}	19.58 ± 2.26Aa^{ns}	4.64 ± 0.26Aa^{ns}	6.67 ± 0.53Aa^{ns}
200 g Se ha^{-1} - UREA	2.36 ± 0.11Aa^{ns}	5.32 ± 0.29Aa^{ns}	16.97 ± 0.62Aa^{ns}	4.21 ± 0.33Aa^{ns}	6.44 ± 0.15Aa^{ns}
50 g Se ha^{-1} - MAP	2.42 ± 0.31Aa^{ns}	5.73 ± 0.52Aa^{ns}	20.29 ± 1.33Aa^{ns}	4.02 ± 0.19Aa^{ns}	5.81 ± 0.40Aa^{ns}
100 g Se ha^{-1} - MAP	2.22 ± 0.44Aa^{ns}	5.79 ± 0.27Aa^{ns}	16.76 ± 0.56Ab^{ns}	4.50 ± 0.12Aa^{ns}	5.82 ± 0.13Aa^{ns}
200 g Se ha^{-1} - MAP	1.74 ± 0.35Aa^{ns}	5.27 ± 0.33Aa^{ns}	17.29 ± 0.56Ab^{ns}	4.01 ± 0.22Aa^{ns}	5.97 ± 0.35Aa^{ns}
50 g Se ha^{-1} - UREA + MAP	2.16 ± 0.37Aa^{ns}	5.15 ± 0.36Aa^{ns}	17.38 ± 1.08Aa^{ns}	4.72 ± 0.24Aa^{ns}	5.67 ± 0.32Aa^{ns}
100 g Se ha^{-1} - UREA + MAP	2.11 ± 0.07Aa^{ns}	5.54 ± 0.33Aa^{ns}	15.14 ± 0.62Aa^{ns}	4.81 ± 0.27Aa^{ns}	5.96 ± 0.46Aa^{ns}
200 g Se ha^{-1} - UREA + MAP	1.48 ± 0.20Aa^{ns}	5.08 ± 0.18Aa^{ns}	18.61 ± 0.90Aa^{ns}	3.92 ± 0.43Aa^{ns}	6.39 ± 0.19Aa^{ns}

B = boron, Cu = copper, Fe = iron, Mn = magnesium, and Zn = zinc. Uppercase letters compare the means of Se-enriched fertilizers within each Se rate and lowercase letters compare the means of Se rates at each Se-enriched fertilizer condition (urea, MAP, or urea + MAP), according to Scott-Knott's test ($p \leq 0.05$). "ns" and "*" indicate non-significant and significant differences, respectively, of the control treatment compared with the others bars by the Dunnett's test ($p \leq 0.05$).

3.7 Selenium amounts remaining in the soil after harvesting

Considering the production of sweet potato biomass at the end of the crop cycle and based on Se contents found in it, we calculated the amounts of Se, in g ha^{-1}, that was left in the soil after harvesting (Table 3). As expected, Se remaining in the soil after harvesting increased upon enhancing Se rates applied, and the higher Se content in the soil resulted with the highest Se rates irrespectively of Se-enriched fertilizer, accounting on average for nearly 7 g ha^{-1} of Se.

Table 3. Estimated levels of residual Se (g ha^{-1}) that remained in the soil after harvesting

Se rates	Se content (mg kg^{-1} DM)	Shoot (kg ha^{-1} DM)	Se residual (g ha^{-1})
Control	0.033	9,066	0.300
50 g Se ha^{-1} - UREA	0.142	10,199	1.456 Ac*
100 g Se ha^{-1} - UREA	0.341	11,101	3.785 Ab*
200 g Se ha^{-1} - UREA	0.626	11,425	7.095 Aa*
50 g Se ha^{-1} - MAP	0.158	11,050	1.745 Ac*
100 g Se ha^{-1} - MAP	0.240	11,012	2.678 Bb*
200 g Se ha^{-1} - MAP	0.592	12,316	7.310 Aa*
50 g Se ha^{-1} - UREA + MAP	0.206	10,778	2.226 Ac*
100 g Se ha^{-1} - UREA + MAP	0.351	11,999	4.186 Ab*
200 g Se ha^{-1} - UREA + MAP	0.622	10,785	6.732 Aa*

DM = dry matter. Lowercase letters compare the treatment means, according to Scott-Knott's test ($p \leq 0.05$). Uppercase letters compare the means of Se-enriched fertilizers within each Se rate and lowercase letters compare the means of Se rates at each Se-enriched fertilizer condition (urea, MAP, or urea + MAP), according to Scott-Knott's test ($p \leq 0.05$). "ns" and "*" indicate non-significant and significant differences, respectively, of the control treatment compared with the other bars by the Dunnett's test ($p \leq 0.05$).

4 DISCUSSION

4.1 Selenium adsorption and desorption

The amount of adsorbed Se increased in response to increasing Se rates added in solution (Fig. 1), in agreement with several other studies evaluating Se adsorption in tropical soils (Abreu et al., 2011; Lessa et al., 2016; Gabos et al., 2014). Also, Se is less retained on sandy soils compared with clayey soils, with its availability being lower upon increasing clay percentage in the soil (Abreu et al., 2011). Selenate is mostly retained in tropical soil components by outer-sphere complexes, which explains its lower sorption and higher mobility compared to selenite (McBride, 1994), which forms an inner-sphere complex with Fe and Al oxides that are commonly found in Brazilian tropical soils (Araujo et al., 2018). Thus, the efficiency of Se biofortification is higher when selenate is applied to soils compared with selenite. For this reason, this study focused on evaluating the sorptive capacity of Se(VI) in the soil of the experimental area before sweet potato cultivation.

Several studies have reported that the presence or fertilization with P in soils may reduce Se adsorption capacity (Goh et al., 2004; Nakamaru et al., 2006). However, there are limited studies addressing the capacity of P and N for Se desorption in tropical soils, as shown in this study (Fig. 2). The addition of P rates (mg L^{-1}) in solution significantly increased Se desorption (Fig. 2a), when P was added as P salt (sodium phosphate), with this increase being not significant when P was added as MAP. Our results differed from those described by dos Santos et al. (2022) who reported that P rates added in solution did not affect the desorbed amounts of selenate in sandy clay loam soil. The fact that MAP did not increase Se desorption may be attributed to a temporary acidic zone generated around the granule when in contact with the soil, because this phosphate fertilizer acidifies the region where their dissolution occurs, favoring Se sorption, and decreasing Se mobility/desorption (McCauley et al., 2009; Guelfi et al., 2022).

The addition of increasing N-urea rates in the solution also increased Se desorption. Contrary to what was reported for P, when applying urea, the pH may have increased around urea granules because urea hydrolysis increases soil pH in the surrounding area of fertilizer granules to nearly 9.0 (Rochette et al., 2009).

The pH plays an important role in the balance between electrical charges in the soil. According to Goh et al. (2004), the amounts of Se adsorbed in soil decrease considerably with increasing pH for both forms of Se. At pH 3, Se adsorbed amounts were 83% for selenite and 46% for selenate, while at pH 7 they were 59% for selenite and 15% for selenate. These variations were attributed to the generation of negative charges in soil colloids by enhancing pH, which caused the repulsion of selenate and selenite anions, thus reducing the adsorption of these two Se species. Also, Goh et al. (2004) reported that the adsorption of both Se species was greatly affected by phosphate due to competition effects. When the sulfate anion was added to solution, only the selenate had its adsorption reduced.

Phosphorus and other competing anions, such as sulfate and nitrate, can directly influence the adsorption of Se in the soil. According to the concentration and competitive interaction between the competing anions and Se, their presence in the soil solution may hinder selenate adsorption, hence decreasing the retention of Se (Natasha et al., 2018).

4.2 Agronomic biofortification of sweet potato with selenium

The average yield of sweet potato (DW) found in this study (30.33 t ha^{-1}) was above the national average of sweet potato yield (14.68 t ha^{-1}; IBGE, 2021). In addition to the high average yield, Se application increased the production of sweet potato shoots and roots dry matter (Figure 3). The response of Se application to plant growth may vary depending on the applied Se-enriched fertilizers. However, in general, several studies have shown positive responses to Se-enriched fertilizers application in food crops grown in tropical soils, such as common beans (Araujo et al., 2022), rice (Premarathna et al., 2012; Félix et al., 2023), soybeans (Silva et al., 2022), and wheat (Stroud et al., 2009; Ramkissoon et al., 2019).

Although Se is not considered an essential element to plants (Hatfield et al., 2014), Se-enriched fertilizers application proved to be a promising strategy to enhance shoot and root Se contents in sweet potato plants without, in general, compromising crop yield. In reality, Se increased sweet potato yield in some cases. Similar results were observed by Félix et al. (2023), where they applied 80 g ha^{-1} Se-enriched urea in different genotypes of rice plants under field conditions. Increases in grain yield were also reported by Silva et al. (2022) applying 80 g ha^{-1} of conventional and enhanced-efficiency MAP enriched with Se in different genotypes of soybean plants under field conditions.

The increase in Se contents (Fig. 4) found in sweet potato in response to the application of Se-enriched fertilizers tested in this present study agrees with the results observed in the desorption test, which showed that Se desorbed amounts increased upon increasing the addition of P-salt and N-urea. Among the Se sources assessed in this study, the higher effectiveness for biofortification was achieved by Se-enriched urea. Namorato (2019) also worked with Se-enriched urea and found positive responses of common bean genotypes grown in tropical soil to applications of 48 g ha^{-1} of Se-enriched urea, where Se contents ranged from 1.14 and 1.71 mg kg^{-1}. Additionaly, Se contents varying from 0.67 to 2.30 mg Se kg^{-1} were also obtained by Félix et al. (2023) in common bean genotypes in response to the application of 80g ha^{-1} of Se-enriched urea.

Ramkissoon et al. (2019) carried out a pot trial to evaluate Se application methods for biofortifying wheat plants using three Australian soils. Applying Se rates equivalent to 10g ha^{-1} of Se-enriched urea, the authors reported Se contents ranged from 0.1 to 0.26 mg kg^{-1}. Moreover, when comparing the efficiency of different Se-enriched fertilizers for common beans biofortification, Araujo et al. (2022) reported higher Se contents in grains when Se-enriched urea was applied.

Selenium contents found in sweet potato plants might be related to the selenate amounts available for absorption. Generally, the absorption and accumulation of Se in the edible parts of food crops are related to Se contents found in the soil. However, soil Se availability is constantly influenced by characteristics such as pH (Goh et al., 2004; Schiavon et al., 2020), the presence of competing ions such as sulfate and phosphate (Lessa et al., 2016; dos Santos et al., 2022), soil texture (Araujo et al., 2018), organic matter (Li et al., 2017), and the presence of microorganisms (Gregorio et al., 2006).

Once absorbed from the soil, Se is mainly transported by the xylem, and the ability to absorb and accumulate Se varies between species, stage of growth, form of the element available in the soil solution, and the presence of competing anions (Gupta and Gupta, 2017). In our study, sweet potato roots showed higher Se content compared with shoots, which may be explained due to the fact that selenate is more easily transported through the phloem when compared with the xylem (Carey et al., 2012).

The highest Se content in sweet potato roots was recorded by applying Se-enriched urea at 200 g ha^{-1}. These results showed that biofortifying sweet potato via the use of Se-enriched fertilizers may be an efficient strategy for increasing Se contents in the edible parts of the plant and consequently enhancing Se intake by population since 34.4 µg person^{-1} day^{-1} of Se (Fig. 5a) could be ingested by consuming sweet potato from this treatment (Se-enriched urea at 200 g ha^{-1}). The values of Se intake presented in this study were estimated taking into account the consumption of 156 g of fresh sweet potato per day (Horodyska et al., 2021) and are relevant to enhance the natural Se intake of the population, since the recommended Se intake of an adult is 70 µg person^{-1} day^{-1} (Kipp et al., 2015) and other agricultural foods should be consumed to provide more Se.

Selenium removal after applying Se-enriched urea at 200 g ha^{-1} was 1.76-fold higher than Se-enriched MAP and Se-enriched MAP + urea at the same rate (Fig. 5b).

This can be attributed to the fertilizer source itself as well as due to the time of application since Se-enriched urea was top-dressing applied in advanced crop stage while Se-enriched MAP was applied at transplanting. There are several factors affecting micronutrients mobilization and translocation within plants, such as type of micronutrient, plant species and phenological stage, environmental conditions, application time, and the presence of other nutrients (White et al., 2004).

The maximum removal found in this study was 3.98 % (Fig. 5b), lower than the average of 12–27% reported by Stroud et al. (2009) and Broadley et al. (2010). A small fraction of Se applied in soils via mineral fertilizers is taken up by plants, where most Se applied retained and immobilized in the soil (Haug et al., 2007), with very little residual effect for subsequent crops (Mathers et al., 2017). This fact is explained by different processes involving the application of Se via soil, such as sorption, leaching, volatilization, and accumulation in other plant organs such as roots, stem, leaves, husk, and others. The Se removal value found in this study is close to what was reported by Lessa et al. (2019), who worked with soil Se fertilization for biofortifying rice plants, reaching the maximum Se removal of 4.45% in polished rice grains.

Selenium amounts that were left in the soil (residual effect) from Se-enriched fertilizers application after sweet potato harvesting were measurable (Table 3), but low, probably due to rapid loss of soluble selenate after application, considering that the soil has lower clay percentage. According to Lessa et al. (2019), soil application of around 40 g ha^{-1} of Se in the form of sodium selenate can guarantee the production of rice grains with recommended levels of Se for human consumption.

Results found in this study emphasized that normal management practices such as the application of phosphate and nitrogen fertilizers can change the availability of Se in tropical soils. As a result, the co-application of Se together with these anions, as Se-enriched fertilizers (containing N and P) showed to be a promising strategy for the biofortification of sweet potato plants.

5 CONCLUSION

Selenium (VI) adsorption capacity of the soil used for growing sweet potatoes increased with increasing Se added in the solution, with the maximum adsorption capacity estimated by the Langmuir model being approximately 45 mg kg^{-1}. Selenate desorbed amounts increased upon increasing P (as a pure salt) and N (as urea) rates, showing that these anions may act to enhance Se availability in the studied soil.

Sweet potato has shown to be an important crop for Se biofortification and responded positively to the application of the tested Se-enriched fertilizers, with Se contents being increased upon increasing Se rates applied, without compromising crop yield. The efficacy of Se-enriched urea for biofortification was higher compared with Se-enriched MAP, agreeing with the desorption results.

Based on sweet potato consumption by Brazilian consumers, the biofortified sweet potato produced in this present study could contribute with a significant fraction of the recommended Se intake by humans (which is 70 µg day^{-1} person^{-1}). By consuming the sweet potato treated with Se-enriched urea at 200 g ha^{-1} (better Se source and with higher Se rate), Se intake estimation can account for 34.4 µg day^{-1} person^{-1} of Se. Future studies are suggested for assessing Se bioaccessibility in those biofortified sweet potatoes produced via the application of Se-enriched fertilizers.

ACKNOWLEDGEMENT

The authors are grateful for the support of the Brazilian National Council for Scientific and Technological Development (CNPq 404412/2021-1 and 141057/2019-0). Also, we thank the Minas Gerais State Research Foundation (Fapemig - APQ-02812-18)), and the Coordination for the Improvement of Higher Education Personnel (CAPES) for financial support. Also, we thank Texas Tech University (Plant and Soil Science Department) and specifically the Environmental Soil Chemistry group for their support mainly in the sorption/desorption studies.

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FINAL CONSIDERATIONS

Agronomic biofortification by applying Se via soil or leaf was effective in increasing Se contents in sweet potato edible parts, improving its nutritional quality. This study has shown that the consumption of sweet potato roots, mainly those that received the highest Se rates (*e.g.,* 200 g Se ha^{-1}), may contribute to providing a considerable amount of Se that is recommended by an adult, which is 70 μg person^{-1} day^{-1}.

Selenium application rates should be different depending on the strategy used for applying Se (*e.g.,* foliar or via soil application). The increase in Se application rates was essential for augmenting Se contents in sweet potato shoots and roots. However, interesting findings that we highlight based on our study are the application of Se-enriched urea via soil and the addition of Zn (together with Se) in foliar treatments, which showed significant improvements in Se biofortification effectiveness. Also, although the biofortification potential with I was much lower than that for Se, the presence of lower Se rates (50 g ha^{-1}) was important for increasing I contents in the plant.

Considering that both studies had similar experimental design (*e.g.,* Se rates) and were carried one next to the other at the same time, it is possible to compare some treatments and state that the biofortification of sweet potatoes with Se proved to be more efficient when carried out via foliar spraying. The values observed for sweet potato yield from the foliar study were comparable/similar to the values found when Se-enriched fertilizers were applied via soil (Fig. 1a). Contrariwise, Se contents determined in sweet potato roots when Se rates were applied as foliar sprays were much higher than those observed with soil Se addition (Fig. 1b).

Finally, we consider that this study contributed to research data involving the biofortification of sweet potato grown in a tropical soil with Se and I (with more focus on Se). However, future studies are needed to evaluate not only agronomic parameters and micronutrient contents, but also soil-plant interactions, nutrient dynamics, and the nutritional values of the biofortified foods produced. Moreover, the observed positive interaction of Se+I at the Se rate of 50 g ha^{-1} and the negative effects observed for higher Se rates justify additional studies testing different combinations of Se+I rates not only in sweet potatoes but also in other relevant food crops, since this interaction (*i.e.*, Se + I) seems to be also very relevant in humans and animals.

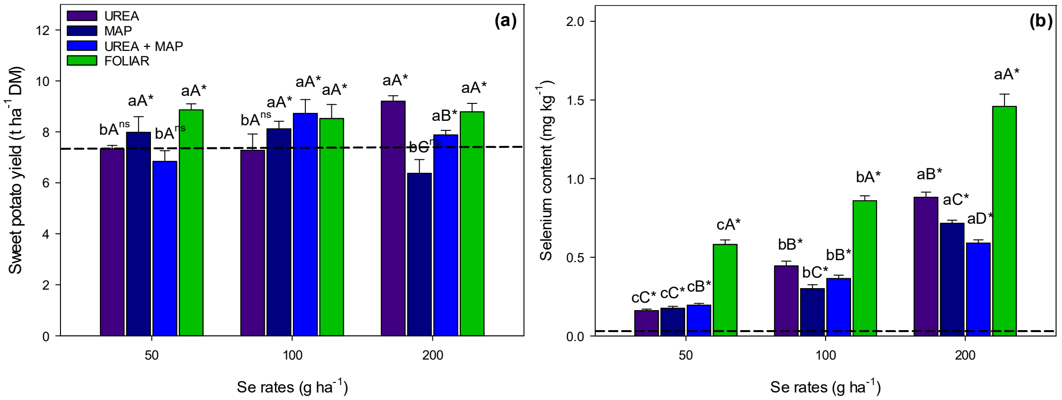

Fig. 1. Production of sweet potato/root dry matter (a), and Se contents in sweet potato roots (b). Uppercase letters compare the means of Se-strategy within each Se rate and lowercase letters compare the means of Se rates at each Se-strategy condition (urea, MAP, urea + MAP, or foliar), according to Scott-Knott's test ($p \leq 0.05$). The dashed line refers to the value found for the control treatment. "ns" and "*" indicate non-significant and significant differences, respectively, of the control treatment compared with the other bars by the Dunnett's test ($p \leq 0.05$). Vertical bars indicate the standard error of average values ($n = 5$).