FABRÍCIO RIBEIRO ANDRADE

SELENIUM IN RICE: BIOFORTIFICATION, PHYSIOLOGICAL ASPECTS AND TOLERANCE TO HYDRIC STRESS

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

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RESUMO GERAL

O selênio (Se) é essencial aos humanos e animais devido às suas propriedades antioxidantes. Embora não seja considerado essencial para as plantas, muitas pesquisas mostram que sua aplicação apresenta efeitos benéficos. Apesar de sua essencialidade aos humanos, o consumo desse elemento pela população brasileira é baixo, principalmente pelas classes com menor poder aquisitivo. Dessa forma, o aumento do Se em alimentos de maior consumo pela população, como o arroz, por meio da biofortificação agronômica, é uma alternativa para atenuar a deficiência desse elemento. Assim, os objetivos deste trabalho, desenvolvido por meio de dois experimentos foram avaliar: a) teor e acúmulo de nutrientes em plantas de arroz com a aplicação de Se; b) o desempenho de plantas de arroz submetidas a condições normais de irrigação e sob déficit hídrico com a aplicação de Se; c) a fotossíntese, produtividade de grãos e o sistema antioxidante das plantas de arroz com a aplicação do elemento; d) o teor de Se nas plantas e grãos de arroz. Os resultados do primeiro experimento demonstram que a aplicação do Se aumenta os teores do elemento na planta, melhora o sistema antioxidante e diminui as espécies reativas de oxigênio; entretanto, não promove ganhos em produtividade. A aplicação foliar é o método mais eficiente para enriquecimento dos grãos de arroz com Se. No entanto, novos estudos devem ser realizados visando à aplicação de doses menores, uma vez que os teores nos grãos encontrados estão acima do recomendado. Há também a necessidade de investigar o efeito residual do Se a partir da aplicação via fertilizante em cultivos subsequentes. Os resultados do segundo experimento indicam que a aplicação de Se reduz os efeitos do estresse hídrico em plantas de arroz por meio de melhorias na fotossíntese, eficiência de uso da água e sistema antioxidante, e ainda aumenta seu teor nos grãos. O estresse hídrico reduz o teor de nutrientes na planta, a produção de biomassa de plantas e grãos e teor de Se nos grãos. No entanto, outros estudos em condições de campo devem ser realizados, uma vez que outros fatores abióticos podem interferir no desenvolvimento das plantas.

Palavras-chave: *Oryza sativa* L. biofortificação agronômica. selenato de sódio.

GENERAL ABSTRACT

Selenium (Se) is essential to humans and animals due to its antioxidant properties. Although not essential to plants, many studies show its beneficial effects. Despite being essential to humans, consumption by Brazilian population is low, especially considering the low-income classes. Thus, the increase of Se through agronomic biofortification in highly consumed foods, such as rice, is an alternative to mitigate the shortage of this element. Therefore, the objectives of this work, which was developed by means of two experiments, were to evaluate: a) content and accumulation of nutrients in rice plants with Se application; b) the performance of rice plants submitted to normal irrigation conditions and under water deficit, with the application of Se; c) photosynthesis, grain yield and antioxidant system of rice plants, with the application of Se; d) the content of Se in rice plants and grains. The results of the first experiment demonstrate that the application of Se increases the levels of the element in the plant, improves the antioxidant system, and decreases oxygen reactive species. However, it does not promote productivity gain. Foliar application is the most efficient method for enriching Se in rice grains. However, studies focused on the application of smaller doses are necessary, given that the contents found in the grains are above recommended levels. It is also necessary to investigate the residual effect of Se application by fertilizer in subsequent crops. The results of the second experiment indicated that the application of Se decreases the effects of water stress on rice plants by improving photosynthesis, water use efficiency and the antioxidant system, in addition to increasing grain content. Water stress reduces nutrient content of the plant, phytomass and grain production, and Se content in grains. However, further studies under field conditions must be conducted, since other abiotic factors may interfere in plants development.

Keywords: *Oryza sativa* L. agronomic biofortification. sodium selenite.

SUMÁRIO

PRIMEIRA PARTE

1 INTRODUÇÃO

A demanda por alimentos cresce anualmente com o aumento da população mundial. De acordo com a Organização das Nações Unidas (ONU), a população mundial irá crescer, em média, 1,10% ao ano e chegará a 9,7 bilhões de pessoas em 2050 (ONU, 2017). No Brasil, estima-se que atualmente a população é de 207 milhões e, em 2050, serão 228,4 milhões de brasileiros (IBGE, 2017). Embora a produção mundial de alimentos seja suficiente para alimentar toda a população mundial, mais de três bilhões de pessoas em todo o mundo sofrem com algum tipo de carência alimentar, como a desnutrição ou fome (RIOS et al., 2009). Os índices de subnutridos foram reduzidos na Ásia, na América Latina e nas Ilhas do Caribe, porém, continuam crescentes na África, mais precisamente, África subsaariana e regiões leste e norte desse mesmo continente (WELCH; GRAHAM, 2004).

Sabe-se que 22 elementos minerais são considerados essenciais para o ideal funcionamento do metabolismo dos seres humanos (WELCH, 2002; WELCH; GRAHAM, 2004; WHITE; BROADLEY, 2005). A deficiência de micronutrientes, incluindo elementos-traço e vitaminas, atinge três bilhões de pessoas em todo o mundo, principalmente nos países em desenvolvimento (DAVEY et al., 2009; FORDYCE, 2013; JONES et al., 2017). A deficiência de selênio (Se) é considerada um grande problema de saúde para 0,5 a 1 bilhão de pessoas em todo o mundo (COMBS JUNIOR, 2007; HAUG et al., 2007).

Em humanos, o Se possui uma estreita faixa entre a deficiência (< 40 μg dia^{-1}) e a toxidez (> 400 μg dia^{-1}) alimentar. Nesse sentido, as agências internacionais, estabeleceram valores de referência dietética na faixa de 30-55 μg dia^{-1} para a ingestão de Se (LEVANDER et al., 2006; WINKEL et al., 2012). Segundo Ferreira et al. (2002), a concentração de Se nos alimentos de origem

vegetal consumidos no Brasil é considerada baixa em relação aos padrões internacionais. Dentre as principais causas está o baixo teor de Se nos solos agrícolas (CARVALHO, 2011; GABOS et al., 2014) e variedades vegetais analisados com baixa capacidade de absorver e acumular esse nutriente na parte comestível. Ainda conforme os autores, o baixo consumo de produtos de origem animal, sobretudo, peixes, pela população de baixo poder aquisitivo, torna essa faixa da população brasileira propensa a apresentar maior deficiência de Se. Adicionalmente, ressalta-se que os peixes podem acumular em seus tecidos grandes quantidades de metais não essenciais (tóxicos ao metabolismo humano) por estarem em contato com água contaminada e outros (YILŴAZ et al., 2010), com isso, minimizando os efeitos benéficos do seu consumo como fonte de Se.

O Se no organismo humano encontra-se normalmente incorporado a proteínas denominadas selenoproteínas, estando intimamente relacionado às complexas funções enzimáticas e metabólicas, sendo um nutriente de grande importância no desenvolvimento infantil, fertilidade e prevenção de uma variedade de doenças (VIARO; VIARO; FLECK, 2001; ALMONDES et al., 2010; WROBEL; POWER; TOBOREK, 2016). Cerca de vinte e cinco selenoproteínas, muitas das quais têm suas funções desconhecidas, foram identificadas em mamíferos (RAYMAN, 2000; KRYUKOV et al., 2003).

Ademais, a associação do Se com a enzima glutationa peroxidase promove a função mais conhecida do Se, que é a de antioxidante mineral, atuando na proteção dos tecidos celulares contra os danos causados pelos radicais livres, promovendo melhor atuação do sistema imunológico, sendo capaz de reduzir o risco de infecções por vírus e se mostrando capaz de tornar mais lento o avanço de diversos tipos de câncer (RAYMAN, 2000; KRYUKOV et al., 2003; TINGGI, 2008; FACOMPRE; EL-BAYOUMY, 2009; MÉPLAN; HESKETH, 2012). Assim, além de atuar na desintoxicação do peróxido de hidrogênio e de outros peróxidos orgânicos, a glutationa peroxidase atua

também na manutenção de grupos sulfidrilos vitais na forma reduzida, na síntese de hormônios derivados do ácido araquidônico e no metabolismo de compostos estranhos ao organismo humano (ALLAN; LACOURCIERE; STADTMAN, 1999; VIARO; VIARO; FLECK, 2001; FERREIRA et al., 2002; RAYMAN, 2002).

O Se tem uma distribuição irregular na crosta terrestre, sendo considerado escasso, e seu teor no solo depende do material de origem e regime hídrico de cada região (HAUG et al., 2007; NANCHARAIAH; LENS, 2015). Em muitas regiões do Brasil, os produtos agrícolas têm baixos teores de Se e isso se deve provavelmente às baixas concentrações do elemento em solos brasileiros (CARVALHO, 2011; GABOS et al., 2014; REIS et al., 2017). Nesse sentido, Silva Júnior et al. (2017) verificaram em castanha-do-brasil, espécie reconhecida como uma das principais fontes naturais de Se, que o teor desse elemento nas castanhas, depende da sua concentração no solo. Outro fator que influencia o teor de Se nos alimentos está relacionado à dinâmica de disponibilidade do Se no solo, a qual pode oscilar, uma vez que alguns estudos têm demonstrado que as formas e o comportamento do Se nos solos variam de acordo com o potencial redox, pH, óxidos/hidróxidos (principalmente de Fe, Al e Mn), argila, matéria orgânica, sistemas de manejo e outros íons (WANG; CHEN, 2003; LESSA et al., 2016; JONES; WINKEL, 2017; REIS et al., 2017).

Dessa forma, há necessidade de se adotar estratégias para aumentar a ingestão de Se. Dentre elas, a biofortificação agronômica constitui numa alternativa viável para inserir o Se na alimentação da população, como tem sido praticada com êxito na Finlândia e Nova Zelândia pela adição do elemento aos fertilizantes inorgânicos (EUROLA et al., 1989; HARTIKAINEN, 2005; LYONS et al., 2005). No Brasil, o Ministério da Agricultura, Pecuária e Abastecimento publicou a Instrução Normativa - nº 46, a qual definiu que os

fertilizantes não devem conter valores de Se inferiores a 30 ppm kg^{-1} (BRASIL, 2016).

Muitos estudos têm mostrado que a aplicação do Se acarreta a elevação do seu teor nas partes comestíveis de alface (MALORGIO et al., 2009; RAMOS et al., 2010; SMOLEŃ; KOWALSKA; SADY, 2014; GOICOECHEA et al., 2015), tomate (PEZZAROSSA et al., 2014; BUSINELLI et al., 2015), crucíferas (SAFFARYAZDI et al., 2012; ÁVILA et al., 2014; FERNANDES; BERTON; COSCIONE, 2014; BAÑUELOS et al., 2015; BACHIEGA et al., 2016), arroz (BOLDRIN et al., 2012, 2013; ZHANG et al., 2014), trigo (BROADLEY et al., 2010; GALINHA et al., 2014; SHARMA; GUPTA; SINGH, 2016) e milho (CHILIMBA et al., 2012; LONGCHAMP et al., 2015).

A cultura agrícola a ser biofortificada exerce grande influência para o sucesso da prática, devendo-se optar por aquelas com maior consumo pela população. Nesse sentido, o arroz apresenta elevado potencial por fazer parte da dieta básica, de pelo menos, dois terços da população mundial, ser cultivado e/ou, consumido em todos os continentes e, segundo estimativas, até 2050 a produção mundial deverá dobrar para que possa atender a demanda desse alimento pela população (GUIMARÃES et al., 2006).

Apesar de o Se ser essencial para os humanos e animais, ainda não foi comprovada sua essencialidade às plantas. Muitas pesquisas mostram que sua presença em baixas concentrações nas plantas apresenta efeitos benéficos. O Se pode aumentar o crescimento, minimizar efeitos de estresses abióticos (seca, salinidade, altas temperaturas e metais pesados) e melhorar o estado nutricional de plantas vasculares (TERRY et al., 2000; GRAHAM et al., 2007; DJANAGUIRAMAN; PRASAD; SEPPANEN, 2010; HASANUZZAMAN; HOSSAIN; FUJITA, 2011; KUMAR et al., 2012; NAWAZ et al., 2015; JIANG et al., 2017). Ramos et al. (2011) e Boldrin et al. (2012, 2013) verificaram um aumento na produção de fitomassa e teor de nutrientes em alface e grãos em

arroz com a aplicação de Se. Seregina et al. (2001) e Seregina & Nilovskaya (2002) constataram maior eficiência do Se na formação do grão de trigo, maior altura de plantas e maior tolerância à seca. Nawaz et al. (2015) verificaram em plantas de trigo submetidas ao estresse hídrico, que a aplicação de Se promoveu a manutenção do turgor foliar e fotossíntese, ocasionando melhorias na absorção de nutrientes e produção.

O Se apresenta importante proteção antioxidante em plantas (DJANAGUIRAMAN et al., 2005). Dependendo da dose utilizada, o Se pode ativar algumas enzimas como a dismutase de superóxido (SOD), catalase (CAT), redutase da glutationa (GR), peroxidase de guaiacol (GOPX) e peroxidase de ascorbato (GPX), que reduzem a formação de peróxido de hidrogênio, reduzindo a taxa de peroxidação lipídica nas células de tecido vegetal, o que resulta em menor senescência (DJANAGUIRAMAN et al., 2005). Ramos et al. (2010, 2012) verificaram o aumento na atividade das enzimas SOD e CAT, acompanhado da redução da peroxidação lipídica em plantas de alface e braquiária com aplicação de pequenas doses de Se na forma de selenato de sódio. A aplicação foliar de selenato de sódio (50 ppm) em soja aos 78 dias após emergência aumentou a produtividade e diminuiu a degradação de clorofilas durante o ciclo da cultura, obtendo-se uma área fotossinteticamente ativa por maior período (DJANAGUIRAMAN et al., 2005).

Embora muitos estudos envolvendo a aplicação de Se em plantas nas últimas décadas tenham sido desenvolvidos pelo mundo, no Brasil, os estudos ligados à aplicação desse elemento ainda são incipientes. É por essa razão que as pesquisas que envolvam a aplicação desse elemento em condições de campo ou que demonstrem os efeitos benéficos de sua aplicação em plantas têm elevada relevância, tanto para a implantação de um programa de biofortificação, quanto para minimizar os prejuízos decorrentes de estresses abióticos. Nesse contexto se inserem os objetivos deste trabalho, os quais foram avaliar: a) teor e acúmulo

de nutrientes em plantas de arroz com a aplicação de Se; b) o desempenho de plantas de arroz submetidas a condições normais de irrigação e sob déficit hídrico com a aplicação de Se; c) a fotossíntese, a produtividade de grãos e o sistema antioxidante das plantas de arroz com a aplicação do elemento; d) o teor de Se nas plantas e grãos de arroz.

A tese está dividida em dois capítulos apresentados na forma de artigos já submetidos, corrigidos conforme sugestões de revisores e corpo editorial e em análise final para a publicação em revistas científicas. O primeiro capítulo, intitulado *Agronomic biofortification of upland rice: effects of selenium dose and application method*, envolveu formas de aplicação e doses de Se em arroz de terras altas sob condições de campo. No segundo capítulo, com o título *Selenium protects rice plants from water deficit stress*, foi avaliado o efeito de doses de Se em plantas de arroz sob estresse hídrico e irrigadas.

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SEGUNDA PARTE – ARTIGOS

ARTIGO 1

Agronomic biofortification of upland rice: effects of selenium dose and application method

(Artigo submetido ao *Journal of Food Composition and Analysis*)

Agronomic biofortification of upland rice: effects of selenium dose and application method

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Abstract

This study aimed to assess the optimal dose and application method to increase selenium (Se) levels in rice grain, focusing on agronomic biofortification. The experimental design was a randomized block, 3 x 5 factorial scheme, using three application methods (applied with fertilizer at sowing, applied via seed treatment and foliar application) and five doses of Se (0, 100, 200, 300 and 400 g ha^{-1} of Se) with three replications. We observed interaction between the application method and Se dose for the chlorophyll index, shoot Se accumulation and Se content in leaves and grains. Foliar application provided the highest values of Se accumulation in biomass and Se content in rice grain. The lipid peroxidation rate and hydrogen peroxide content decreased up to a 200 g ha^{-1} dose, whereas superoxide dismutase (SOD, EC 1.15.1.1) and ascorbate peroxidase (APX, EC 1.11.1.11) enzyme activities increased in response to Se application. Selenium application stimulated the antioxidative system, reducing the reactive oxygen species and increasing Se content linearly in plants and grain. This study provides useful information for agronomic biofortification of upland rice to improve human health.

Keywords: *Oryza sativa* L.; biofortification; food analysis; food composition; application methods; human health; selenate; foliar application; antioxidant enzymes

Abbreviations: APX, ascorbate peroxidase; CAT, catalase; GR, reductase glutathione; H_2O_2, hydrogen peroxide; MDA, malondialdehyde; SOD, superoxide dismutase; SPAD, soil plant analysis development; USEPA, United States Environmental Protection Agency; WHO, World Health Organization

1. Introduction

The lack of one or more essential nutrients during the critical stages of human growth can interfere with development. According to the World Health Organization (WHO), this is a problem that affects both developing and underdeveloped countries (White, 2016). Selenium (Se) is an essential nutrient for humans and animals due to its antioxidant properties. This nutrient is involved in a number of chemical reactions, such as protecting tissue from free radicals, improving the immune system, controlling hormone metabolism, acting on cardiovascular diseases and slowing the progression of cancer (Fairweather-Tait et al., 2011; Rayman et al., 2012; Gharibzahedi and Jafari, 2017). In this context, agronomic biofortification with Se has high potential to provide continuing benefits to developing countries over time due to lower costs for post-harvest supplementation and fortification (White and Brown, 2010).

The appropriate level of dietary Se uptake in a population is highly correlated with the Se content in its food resources (Combs Junior, 2001). Global research investigating the content of Se in rice (*Oryza sativa* L.) has shown low concentrations of this element in most samples (Williams et al., 2009). Rice, characterized as a staple food for more than half of the world's population, is the second most cultivated food in the world, with an annual production of approximately 741 million tons. Brazil contributes with 12 million

tons of rice annually (1.62% of world production) and stands out as the only non-Asian country among the 10 largest producers (FAOSTAT-FAO, 2016).

Studies involving the application of Se in rice cultivation aimed at reducing Se deficiencies in the world's population have high potential since the consumption of rice as a staple food is well established on all continents, including regions of high population density where rice is the main source of nutrients (Fageria et al., 2003; Abilgos-Ramos et al., 2007). In Brazil, there are few studies involving biofortification of rice with Se, and they have been restricted to controlled environments (Boldrin et al., 2013, 2012; Fernandes et al., 2014). Available studies have shown that the content of Se in grains increases with the application of Se in the form of sodium selenate.

In Brazil, the content of Se in soil is considered low, with average values approximately 0.19 mg kg^{-1} (Gabos et al., 2014). This is reflected in low levels in plants and consequently in the foods consumed by the population (Ferreira et al., 2002; Maihara et al., 2004). Since 1984, the Finland government has been adding sodium selenate to commercial fertilizers in response to low Se content in the soil and thus the population's diet (Varo et al., 1988). However, agronomic fortification of plants via foliar application of Se is rare. According to Boldrin et al. (2013), in greenhouse, the form of Se application influences its absorption, as soil application can increase the content of this element in rice grains by up to 450% compared to foliar application.

Agronomic biofortification is an excellent process by which the quality and the content of nutrients in rice (e.g., Se) can be increased. The role of Se in humans and animals is making special proteins, called antioxidant enzymes that play a role in protecting the body from the damaging effects of heavy metals, free radicals and other harmful substances (Gharibzahedi and Jafari, 2017).

Thus, field studies to determine the appropriate Se dose and the application method have high relevance to the implementation of rice biofortification program. Therefore, this study aimed to evaluate the effects of different forms of application and doses of sodium selenate on the growth, grain yield, antioxidant enzyme activity, Se content and Se accumulation in upland rice cultivated under field conditions.

2. Material and methods

2.1. Chemicals, enzymes and reagents

All the chemicals and reagents used were of analytical grade of the highest purity. Sodium selenate (Na_2SeO_4, purity ≥99%), nitric acid (HNO_3, concentration 70%), white clover (trace elements) (BCR 402), poly(vinylpolypyrrolidone) (PVPP), β-nicotinamide adenine dinucleotide 2-phosphate reduced tetrasodium salt hydrate (NADPH, purity ≥93%), ascorbic acid (AA, purity ≥99%), nitrotetrazolium blue chloride (NBT, purity ≥98%), methionine (MetH, purity ≥98%), riboflavin (RB, purity ≥98%), hydrogen

peroxide solution (H_2O_2, concentration 30%), oxidized glutathione (GSSG, purity ≥98%), trichloroacetic acid (TCA, purity ≥99%), potassium iodide (KI, purity ≥99%) and thiobarbituric acid (TBA, purity ≥98%) were purchased from Sigma-Aldrich (Saint Louis, USA). Potassium phosphate anhydrous monobasic (K_2HPO_4) with potassium phosphate anhydrous dibasic (KH_2PO_4) (PP) and ethylenediaminetetraacetic acid (EDTA) were purchased from Labsynth (Diadema, São Paulo, Brazil). The determination of the enzymes ascorbate peroxidase (APX, EC 1.11.1.11), superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6) and glutathione reductase (GR, EC 1.8.1.7), as well as of hydrogen peroxide (H_2O_2) and lipid peroxidation, was performed in an Epoch Microplate Spectrophotometer (BioTek Instruments, Winooski, Vermont, USA).

2.2. Experimental design and rice cultivation

The experiment was carried out from January to May 2014 at the experimental farm of the Federal University of Lavras (Lavras, MG), which is located at latitude 21°13'14'' S; longitude 44°48'55" W, with an altitude of approximately 900 m. The soil was an Ultisol that had been fallow for two years. Soil physical and chemical analyses at the 0-0.20 m layer followed the methods described by Embrapa (1997). The results of soil characterization are shown in Table 1. The content of Se was 0.3 mg kg^{-1}, as determined after soil digestion according the USEPA method 3051A (USEPA, 1998). The precipitation and

temperature data recorded during the experimental period are shown in Fig. 1. Due to unstable rainfall during the study period, additional irrigation, using a sprinkler system, was systematically applied every two days without rainfall to keep the soil as humid throughout the experiment, as adopted by Santos et al. (2002).

The experimental design was a randomized block with a 3 x 5 factorial scheme, using three application methods (applied with fertilizer at sowing, applied via seed treatment and foliar application) and five doses of Se (0, 100, 200, 300 and 400 g ha^{-1} of Se), with in three replications. Na_2SeO_4 was used as the Se source due its higher potential for Se uptake by plants (Boldrin et al., 2012). Each plot was composed of 8 lines 3 m in length, spaced by 0.25 m, totaling an area of 6 m^2. Fertilization was carried out with 300 kg ha^{-1} of 08-24-12 (N-P-K) fertilizer – applied during planting, and 55 kg of N applied in cover 45 days after planting.

The upland rice cultivar used in the experiment was BRSMG Curinga, which is considered medium cycle and of the long fine class and is recommended for rainfed and floodplain areas. Seeding was carried out on January 24, 2014, using 85 kg ha^{-1} of seeds that had previously been treated with tricyclazole fungicide (Bim 750 BR®) at a dose of 225 g active ingredient (a.i.) per 100 kg^{-1} of seeds together with imidacloprid and thiodicarb insecticide (Cropstar®) at the dose of 150 ± 450 g a.i. 100 kg^{-1} of seeds. Prior to sowing, the

herbicide glyphosate (Roundup®) was applied at a dose of 1,080 g a.i ha^{-1} to eliminate cover crops and invasive plants. Post-emergence weed control was applied 25 days after planting, using the herbicide cyhalofop butyl (Clincher®) at a dose of 225 g ha^{-1} a.i.; after 55 days, the weeds were manually eliminated.

The application of Se with fertilizer aimed at adding Na_2SeO_4 to the fertilizer. The seed treatment application method involved the addition of Na_2SeO_4 together with the fungicide and insecticide for the treatment. Foliar application was carried out at the beginning of the floral differentiation phase of the crop using a pressure sprayer pressurized by CO_2 with four XR 110-02 spray tips spaced 0.50 m, applying a volume equivalent to 150 L ha^{-1}.

2.3. Dry biomass

During crop flowering, the height of the plants and the aerial dry biomass were measured using ten plants per plot. The height of the plants was determined by measuring the plant base to the end of the last fully expanded leaf. Dry shoot biomass was obtained after plants were dried in an air oven with forced circulation at 60 °C for 72 h and weighed on a precision scale.

2.4. SPAD index

The soil plant analysis development (SPAD) chlorophyll index was determined during crop flowering whith a SPAD meter (SPAD-502, Minolta,

Osaka, Japan) using the first fully developed leaf from the apex to the base (flag leaf). Six leaves per plot were sampled.

2.5. Productivity

At the end of the crop cycle, five panicles per plot were collected to evaluate the number of grains per panicle. Subsequently, the plants in the plot area were manually collected and harvested. Production in kg ha^{-1} of grains was obtained, standardized at 13% moisture, and the mass of one thousand grains was evaluated.

2.6. Chemical analysis of nitrogen, sulfur, and selenium

During crop flowering, 15 leaves per plot were collected to assess the levels of nitrogen (N), sulfur (S), and Se. The plant material and the peeled grains were oven dried and milled in a standard Wiley mill (40-mesh screen). Subsequently, chemical analyses were performed to determine the N and S contents, according to Malavolta et al. (1997).

The chemical analysis for the determination of Se content in leaf and shoot biomass and grains was performed following USEPA method 3051A (USEPA, 1998), in which 0.5 g of dry tissues and grains was digested in 5 mL of HNO_3 in a microwave oven (CEM, model Mars 5CEM Corporation, Matthews, USA). A sample of standard reference material (White Clover - BCR 402, Institute for Reference Materials and Measurements, Geel, Belgium) for plant

material was included in each digestion batch for quality control purposes. The mean recovery for Se in this standard reference material (white clover) was 97.53% (n = 7). The extracts were analyzed by atomic absorption spectroscopy with electrothermal atomization in a graphite furnace (PerkinElmer, model AAnalyst 800, Midland, Canada).

2.7. Antioxidant enzymes

Five leaflets were collected from each plot during flowering for the biochemical analysis and were immediately conditioned in liquid nitrogen (LN_2) and stored at -80 °C until analysis. Antioxidant enzyme activity was quantified from the leaf extract obtained by maceration of 0.1 g of leaves in LN_2 followed by addition to a buffer solution containing 100 mM PP (pH 7.8), 0.1 mM EDTA (pH 7.0), 10 mM AA and 22 mg PVPP (Biemelt et al., 1998) and centrifugation in a Heraeus Megafuge 40R (Thermo Scientific, Massachusetts, USA) at 12,000 g for 10 min at 4 °C. The supernatant was collected and used in the enzymatic analysis of APX, SOD, CAT and GR.

APX activity was determined by monitoring the oxidation rate of ascorbate at 290 nm for 3 min. For this, an aliquot of the enzyme extract was added to incubation buffer, composed of 100 mM PP (pH 7.0) and 0.5 mM AA, previously incubated at 30 °C. Prior to measuring the oxidation rate, 0.1 mM H_2O_2 (Nakano and Asada, 1981) was added to the sample. The molar extinction coefficient of 2.8 mM^{-1} cm^{-1} was used for calculations.

SOD activity was evaluated by the enzyme's ability to inhibit the photoreduction of NBT as proposed by Giannopolitis and Ries (1977). An aliquot of the supernatant was added to incubation medium composed of 50 mM PP (pH 7.8), 14 mM MetH, 0.1 μM EDTA, 75 μM NBT and 2 μM RB. The tubes with the reaction medium and samples were illuminated for 7 min with a 20 W fluorescent lamp. For the control, the same reaction medium without the sample was illuminated. The readings were performed at 560 nm. One unit of SOD corresponds to the amount of enzyme capable of inhibiting the photoreduction of NBT by 50% under the assay conditions.

CAT was evaluated according to the method described by Havir and McHal (1987) in which an aliquot of the enzyme extract was added to incubation medium containing 100 mM PP (pH 7.0), previously incubated at 30 °C. Immediately prior to absorbance reading, 12.5 mM H_2O_2 was added to trigger the reaction. CAT activity was determined by following the consumption of H_2O_2 at 240 nm for 3 min. The extinction coefficient value of 36 mM^{-1} cm^{-1} was used for calculations.

GR activity was determined based on the decrease in absorbance at 340 nm due to the oxidation of NADPH to NADP according to the method described by Cakmak et al. (1993). The incubation medium, composed of 50 mM PP (pH 7.8) buffer and 1 mM GSSG, was incubated at 30 °C. Thereafter, 75 μM

NADPH was added, and spectrophotometric reading was performed. The extinction coefficient value of 6.22 mM^{-1} cm^{-1} was used for calculations.

2.8. Hydrogen peroxide

Hydrogen peroxide (H_2O_2) was quantified from the extract obtained by maceration of 0.2 g of leaves in LN_2. The extract was then homogenized in 1.5 mL of TCA and centrifuged in a Heraeus Megafuge 40R at 12,000 g for 15 min at 4 °C. Hydrogen peroxide levels were determined by measuring the absorbance at 390 nm. Aliquots of the supernatant were added to the reaction medium containing 2.5 mM PP (pH 7.0) buffer and 500 mM KI (Velikova et al., 2000). The quantification of H_2O_2 was performed based on a standard curve with known concentrations of H_2O_2.

2.9. Lipid peroxidation

Lipid peroxidation was determined by the quantification of thiobarbituric acid reactive species as described by Buege and Aust (1978). A total of 0.2 g of leaves was macerated in LN_2 and 20% PVPP (w/v), which were homogenized in 0.1% (w/v) TCA. The homogenate was centrifuged in a Heraeus Megafuge 40R at 10,000 g for 10 min. Aliquots of the supernatant were added to the 0.5% (w/v) TBA and 10% (w/v) TCA reaction medium and then incubated at 95 °C for 30 min. The reaction was stopped by ice cooling and absorbance was measured in a spectrophotometer at 535 nm and 600 nm. The

concentration of malondialdehyde (MDA) was calculated with the following equation: [MDA] = (A535 - A600) / (ξ X b), where ξ = molar extinction coefficient = $1.56x10^{-5}$ and b = optical length = 1.

2.10. Statistical analysis

In all of the datasets considered, the normality of the data was analyzed using the Anderson-Darling test, and homoscedasticity was analyzed with the variance equation test (or Leven's test). The quantitative factors were submitted to polynomial regression analysis after analysis of variance. In case of significance ($p \leq 0.05$), the qualitative variables were compared by the Scott-Knott test using the statistical R software (version 3.2.3, Institute for Statistics and Mathematics, Vienna, Austria) (RDCT, 2011) and the graphs were made in the Sigma Plot Programme (version 12.5, Systat Software Inc., Chicago, IL, USA).

3. Results

Interaction ($p \leq 0.05$) between the application method and the dose of Se was observed for the SPAD index (Fig. 2A). Foliar content and N and S accumulation in the aerial part of the plants at the time of flowering were influenced ($p \leq 0.05$) only by the Se dose (Fig. 2B, C, D and E). Plant height, shoot dry matter, number of grains per panicle, mass of one thousand grains and grain yield were not influenced by the dose or application method of Se.

There was an increase in the SPAD index with the application of Se, particularly for the application through seed treatment, which promoted the highest values at all doses of Se, except for the 100 g ha^{-1} and 200 g ha^{-1} doses, compared to the other methods (Fig. 2A). The highest SPAD index was observed at the estimated dose of 246.72 g ha^{-1} for the plants that received the fertilizer at the dose of 400 g ha^{-1} for seed treatment and foliar application (Fig. 2A).

Nitrogen and sulfur content and accumulation increased with the addition of Se regardless of the application method (Fig. 2B, C, D and E). The applied dose of 400 g ha^{-1} promoted the highest N content in leaves and accumulation of N and S in plants (Fig. 2B, D and E). The highest content of S in leaves was reached with the estimated dose of 285 g ha^{-1} of Se (Fig. 2C).

Interaction ($p \leq 0.05$) between the application method and the Se dose was observed for the Se content in the leaves and grains and the accumulation of Se in rice plants (Fig. 3A, B and C). The Se content in leaves (Fig. 3A) and grains (Fig. 3C), as well as Se accumulation in rice plants (Fig. 3B), increased with the application of Se doses, especially by foliar application, which promoted the highest values at all doses of Se, except for the foliar content at the dose of 400 g ha^{-1}, which did not differ from the other application methods (Fig. 3A).

The highest Se content in the leaves was observed with the estimated dose of 271 g ha^{-1} for foliar application and the applied dose of 400 g ha^{-1} for the treatment of seeds and application with fertilizer (Fig. 3A). For all application methods, accumulation of Se in rice plants followed a linear regression model, except for the seed treatment method, in which there was no significant effect of the applied Se applied dose (Fig. 3B). Therefore, the dose of 400 g ha^{-1} promoted greater accumulation of Se when it was applied using foliar application and with fertilization.

In grains, the Se content increased linearly with increasing application doses for all application forms (Fig. 3C). The highest concentrations of Se in grain were observed with the application of Se at 400 g ha^{-1}. The Se content of grains at the dose of 400 g ha^{-1} was 2.53 mg kg^{-1} via seed treatment, 2.81 mg kg^{-1} via fertilizer application and 9.23 mg kg^{-1} via foliar application. Among the application methods, foliar application was the most efficient at increasing the Se content in the grain, showing the highest values among the evaluated application methods.

The enzymatic activities of APX and SOD, as well as the lipid peroxidation and the concentration of H_2O_2, were influenced ($p \leq 0.05$) by the Se dose regardless of the application method (Fig. 4A, B, C and D). APX activity increased linearly with increasing Se dose (Fig. 4A). The activity of SOD increased with the application of Se up to 200 g ha^{-1}, after which a decrease was

observed for the highest doses (Fig. 4B). The concentration of MDA and H_2O_2 content decreased with the application of up to 200 and 300 g ha^{-1} of Se, respectively, and increased at the dose of 400 g ha^{-1} of Se (Fig. 4C and D).

4. Discussion

The effect of the application of Se on leaves and grains and on the activity of antioxidant enzymes in rice plants (Figs. 3 and 4) was reported in previous studies on rice (Boldrin et al., 2012, 2013; Fernandes et al., 2014), lettuce (Ramos et al., 2010) and wheat (Poblaciones et al., 2014). An increase in the SPAD index with the addition of Se was shown by the increase in N content in the leaves and biomass (Fig. 2B and D) and by the consequent participation of N as a constituent of the chlorophyll molecule in the photosynthetic process and in the formation of amino acids and proteins (Debaeke et al., 2006). On the other hand, the increase in N content and accumulation (Fig. 2B and D) is related to the application of Se since there is evidence that N can metabolically regulate Se (Jezek et al., 2011; Hajiboland and Sadeghzade, 2014). This may lead to increased uptake and assimilation of N through increased nitrate reductase enzyme activity, as observed by Hajiboland and Sadeghzade (2014) and Xu et al. (2013) in wheat and tobacco plants (*Nicotiana tabacum* L.), respectively. An effect of Se on the SPAD index was observed by Jiang et al. (2015) in tobacco

plants with the application of 6 mg kg^{-1} of Se (sodium selenite) to the soil under greenhouse conditions.

The increase in S content in leaves and its accumulation in biomass with the application of Se (Fig. 2C and E) was due to the synergistic interaction between selenate and sulfate due to their similar chemical properties (White et al., 2004). According to Sors et al. (2005) and Pilon-Smits and Quinn (2010), selenate utilizes the same route of assimilation as S. Therefore, changes in absorption rates and S content in the rice plants promoted by Se were expected. This is the same pattern observed by Ramos et al. (2011) and Hawrylak-Nowak et al. (2015) in lettuce and cucumber plants, respectively. In a study with barley and rice, Mikkelsen and Wan (1990) found that by increasing the concentration of selenate in the culture solution, the S content increased significantly in the aerial part of plants.

The application of Se promoted its increase in content and accumulation in rice plants, presenting different behavior as a function of the application form (Fig. 3A and B). Increases in Se content and accumulation in plants subjected to the application of Se are evident in lettuce (Ramos et al., 2010, 2011; Hawrylak-Nowak, 2013), Brachiaria (Ramos et al., 2012), tomato (Schiavon et al., 2013) and cucumber plants (Hawrylak-Nowak et al., 2015). Increasing the levels of Se in plants and grains without damaging productivity is a key factor in

biofortification programs. In this sense, the doses applied in the present study did not cause a significant reduction in plant biomass or rice yield.

The Se content in rice grains increased significantly when plants were submitted to increasing doses of Se, with an increase of 0.60, 0.69 and 1.98 mg kg^{-1} per 100 g ha^{-1} of Se for the seed, fertilizer and foliar treatments, respectively (Fig. 3C). In Brazil, the per capita consumption of rice is 110 g day^{-1} (FAOSTAT-FAO, 2016). The USDA (2016) recommends a minimum daily intake of Se for adults of 40 μg day^{-1} and cites the maximum tolerable level of Se as 400 μg day^{-1}. The content of Se found in the grains at the dose of 100 g ha^{-1} of Se applied via the seed, fertilizer and foliar treatments provided, on average, levels of 75.90, 84.70 and 288.2 μg Se day^{-1}, respectively. However, further studies are required regarding agronomic biofortification of rice with Se to determine the adequacy of the doses to be applied considering food safety.

The highest Se content was observed in leaves (except for the 400 g ha^{-1} dose). The Se accumulation in plants and higher Se content in grains (Fig. 3A, B and C) observed with foliar application are due to the transport of selenate via the phloem, which is more efficient than that via the xylem (Boldrin et al., 2013). Poggi et al. (2000) observed good mobility of Se in the phloem of potatoes when Se was applied via foliar application. This type of application at the highest dose promoted 3.64 and 3.28 times higher Se content in rice grains compared to applications performed through seed treatment and fertilizer,

respectively (Fig. 3C). Further studies are needed to observe the uptake of Se in crops from each of these forms of application since there is evidence that the application of Se to the soil may have a residual effect on future crops.

Selenium application increased the activity of the antioxidant enzymes APX and SOD, and decreased the H_2O_2 content and lipid peroxidation up to the doses of 200 and 300 g ha^{-1} of Se, respectively (Fig. 4A, B, C and D). These results corroborate the studies conducted by Ramos et al. (2012) in Brachiaria, Hawrylak-Nowak (2013) in lettuce plants and Chu et al. (2013) in wheat plants. The APX enzyme is one of the first to act under oxidative stress, disrupting H_2O_2 into water and oxygen, which leads to a decrease in the content of this reactive oxygen species. Hydrogen peroxide may originate from SOD activity (Fig. 4B), which is responsible for disrupting superoxide into H_2O_2 (Kibinza et al., 2011). However, the increase in its content at the 400 g ha^{-1} dose of Se may be due to increased lipid peroxidation. Chu et al. (2013) verified decreases in lipid peroxidation and H_2O_2 levels in wheat plants with the application of Se. The increase in lipid peroxidation from the application of 300 g ha^{-1} of Se is a consequence of increased H_2O_2 levels and may be related to the pro-oxidant effect of Se at high concentrations (Hartikainen et al., 2000). Although APX activity increased linearly, it was not sufficient to prevent damage to cell membranes.

5. Conclusions

The application of Se at 200 g ha^{-1} promoted the best results for the SPAD index, S and N content and accumulation and activity of APX and SOD enzymes. The highest values of Se accumulation in plants and Se grain content were obtained with the application of 400 g ha^{-1} of Se in all application methods. Selenium application stimulated the antioxidative system and reduced reactive oxygen species in rice plants, but it did not increase grain yield. The most efficient application method to increase Se in plants and grain content was foliar. The lower dose at Se application resulted in Se concentrations in the grains above the daily average recommendation to human consumption. Further studies under field conditions are needed to establish the optimal levels and application methods of Se for agronomic biofortification of rice plants.

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Table 1
Chemical and physical properties of the soil studied.

pH H_2O	P	K	P-rem	Ca	Mg	Al	H+Al	CEC	OC	Clay	Silt	Sand
	--mg kg^{-1}--		mg L^{-1}	----------cmol$_c$ kg^{-1}----------					%	----g kg^{-1}----		
5.8	2.0	96.0	24.79	2.90	1.10	0.00	2.32	6.57	3.28	410	110	480

P and K extracted with Mehlich-1; P-rem - ion exchange resin; Ca, Mg and Al extracted with KCl; H + Al extracted with SMP buffer; CEC – cation exchange capacity; OC – organic carbon.

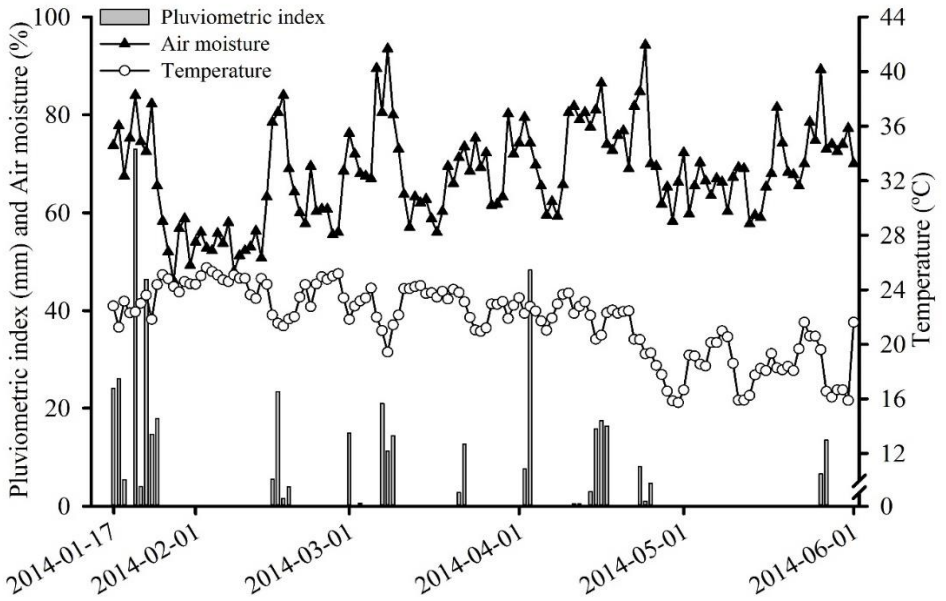

Fig. 1. Pluviometric index, air moisture and average temperature during the experiment period. Lavras-MG, 2014.

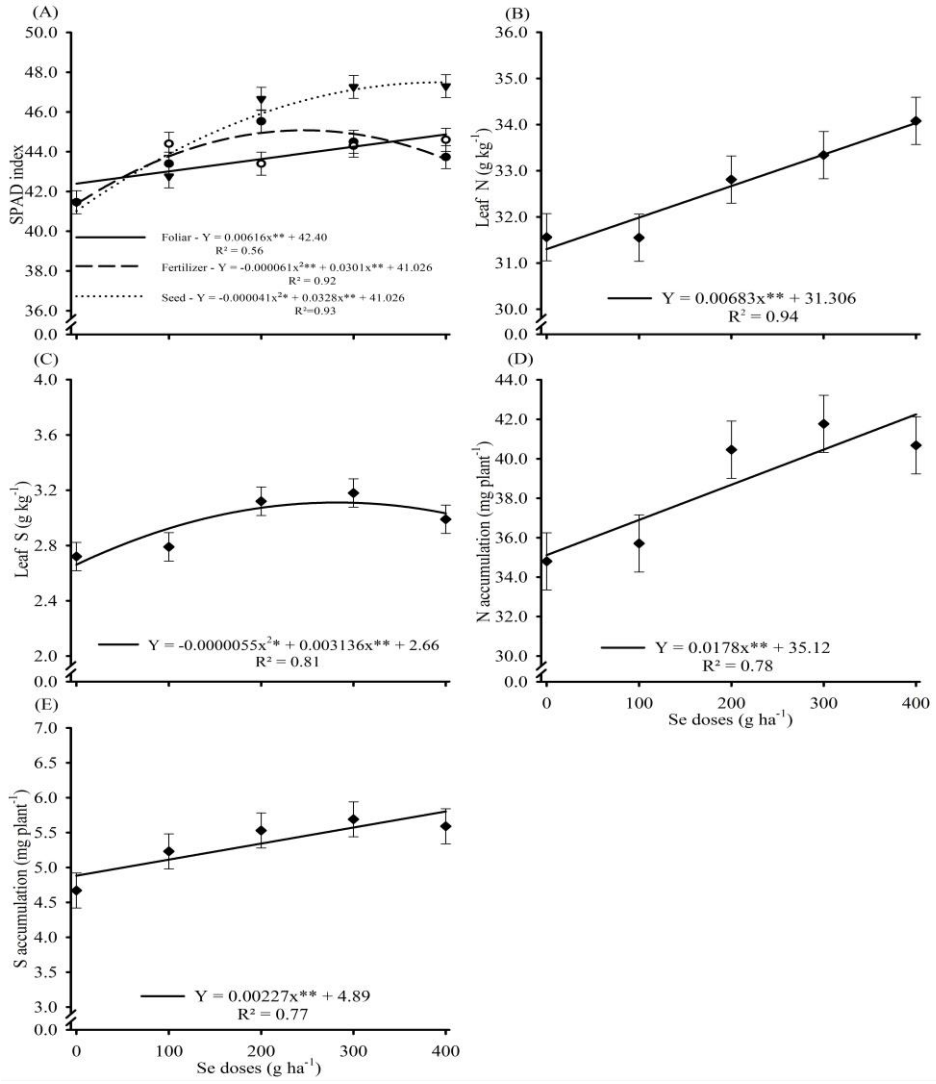

Fig. 2. SPAD index (A), nitrogen content (B), sulfur content (C) and accumulation of nitrogen (D) and sulfur (E) in rice plants at the time of flowering as a function of the application method and selenium (Se) doses. The ** and * represent significance at 1 and 5% probability by Student's t-test, respectively. The vertical bar indicates the standard error of the mean (n = 3).

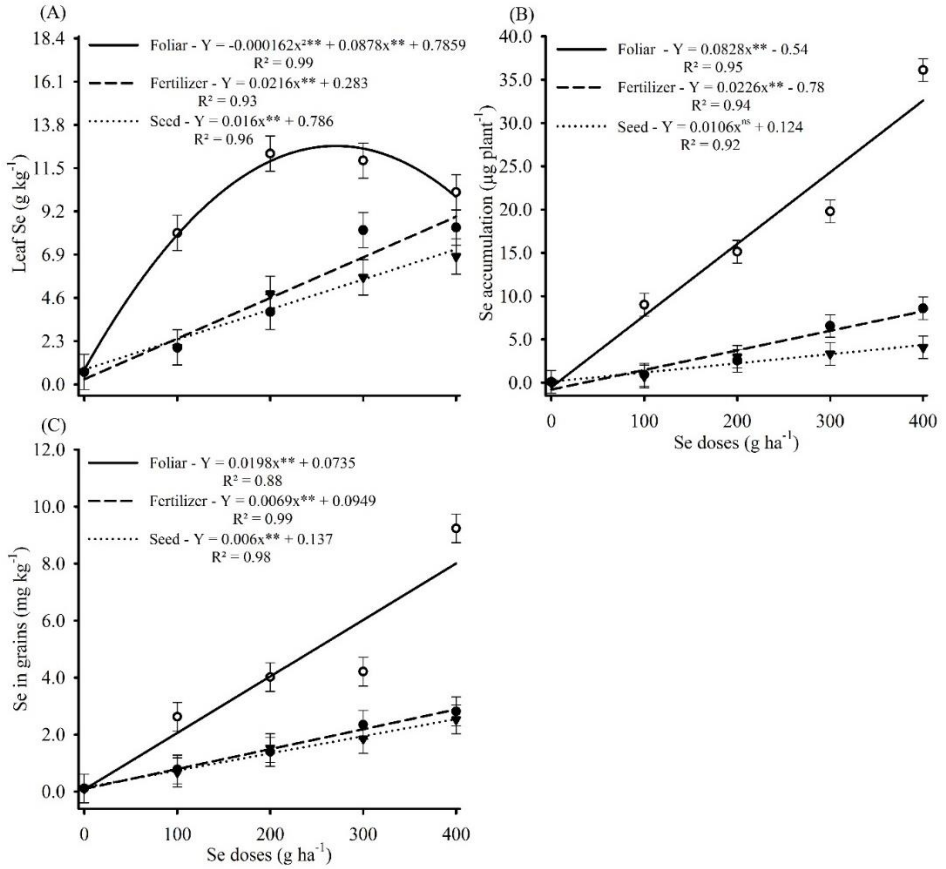

Fig. 3. Leaf content (A), accumulation (B) in plants during flowering and grain content (C) as a function of application method and selenium (Se) doses applied in upland rice. ns: not significant; ** and * represent significance at 1 and 5% probability by Student's t-test, respectively. The vertical bar indicates the standard error of the mean (n = 3).

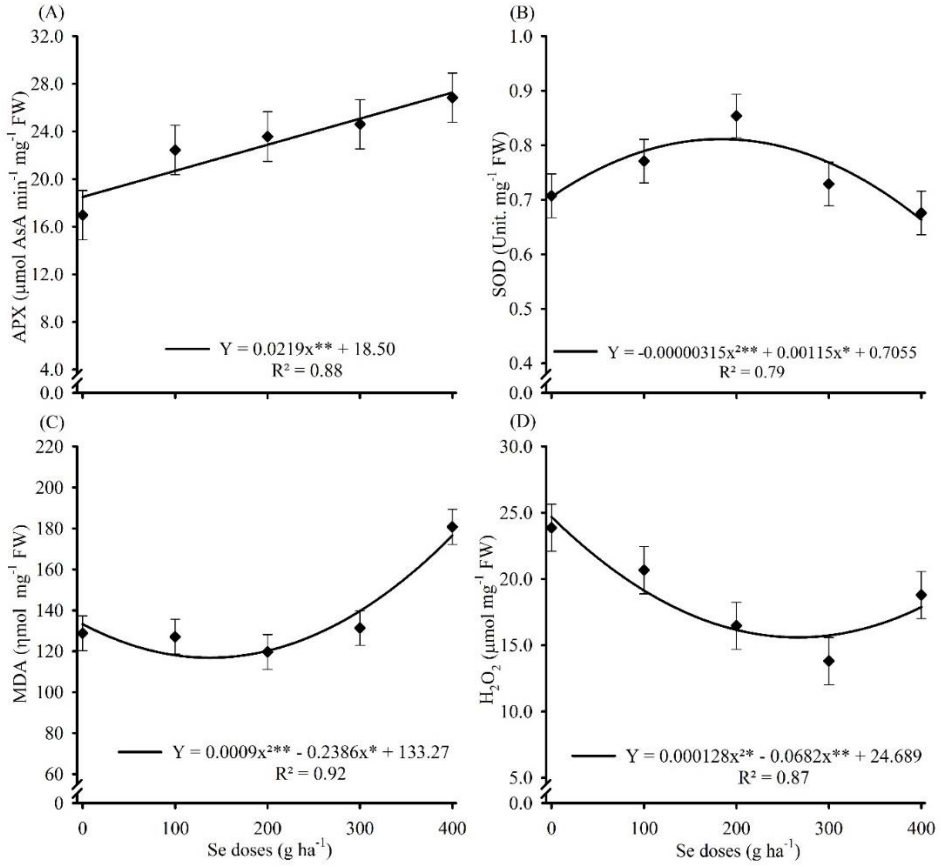

Fig. 4. Effect of the application of Se doses on enzymatic activities of ascorbate peroxidase (A), superoxide dismutase (B), lipid peroxidation (C) and hydrogen peroxide (D) in leaves. The ** and * represent significance at 1 and 5% probability by Student's t-test, respectively. The error bar indicates the standard error of the mean (n = 3).

ARTIGO 2

Selenium protects rice plants from water deficit stress

(Artigo submetido à revista *Plant Physiology and Biochemistry*)

Selenium protects rice plants from water deficit stress

Running title: Selenium reduces ROS and biofortifies rice grains

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Abbreviations: A, CO_2 assimilation rate; APX, ascorbate peroxidase; Ci, internal CO_2 concentration; CAT, catalase; E, transpiration; EiC, instantaneous carboxylation efficiency; FW, fresh weight; gs, stomatal conductance; H_2O_2, hydrogen peroxide; MDA, malondialdehyde; ROS, reactive oxygen species; SOD, superoxide dismutase; WUE, water use efficiency.

Selenium protects rice plants from water deficit stress

Abstract

Selenium (Se) is essential to humans and animals due to its antioxidant properties. Although it is not considered an essential nutrient for plants, many studies show the its presence in low concentrations provides beneficial effects to plants by participating in antioxidant defense systems and enhancing tolerance to abiotic stress. Therefore, this study aimed to evaluate the effects of Se doses on rice plants under different soil water conditions. The experiment was conducted on an Oxisol using four doses of Se (0, 0.5, 1.0 and 2.0 mg kg^{-1}) and two soil water conditions (irrigated and water deficit). Increased Se effects were measured by plant height, SPAD index, sulfur (S) and copper (Cu) accumulation, gas exchange, increases in the activity of superoxide dismutase (EC 1.15.1.1) and decreases in hydrogen peroxide (H_2O_2). The accumulation of Se in biomass and Se grain content increased linearly with the applied doses. Water deficit negatively affected the growth and production of rice plants. A positive effect on rice cultivation was observed, as Se minimized water deficit effects. Se application also increased Se grain content from 0 to 20.78 mg kg^{-1} under water deficit conditions as well as from 0 to 23.58 mg kg^{-1} under irrigated conditions. This study provides useful information about the roles of Se both in

protecting rice plants from water deficit stress and in the agronomic biofortification of upland rice, in which Se content in edible parts increased.

Keywords: agronomic biofortification; sodium selenate; food composition; antioxidant enzymes; abiotic stress

1. Introduction

Cultivated plants are frequently exposed to adverse conditions that affect their growth and productivity. Among the various types of environmental stress, drought is considered the most devastating because of production losses, which can reach billions of dollars annually worldwide (Aon, 2015; Lambers et al., 2008; Tardieu et al., 2014). It is estimated that by 2050, when the world population is expected to reach 9.7 billion people, approximately 49% of the global grain production will be cultivated in regions affected by water deficit (Rosegrant, 2016).

Rice (*Oryza sativa* L.) is the second most cultivated cereal in the world and the main food source for more than half of the world population. Approximately 75% of rice production worldwide comes from planting in an irrigated and/or flooded system (FAO, 2017). However, these cultivated area expansions are limited due to strong impacts on environmental problems (Sander et al., 2014). Therefore, dryland rice cultivation tends to expand into arid

regions, where dry spells are more consistent. These regions are characterized by long periods of soil water deficit due to interruptions in rainfall during the rainy season (Assad and Sano, 1998).

Water deficit reduces soil water potential. This reduction has direct implications for transpiration, photosynthesis, leaf temperature, stomatal opening and antioxidant metabolism, all of which affect the growth, development and especially the yield of economically important crops (Nawaz et al., 2015). The harmful effects on plant metabolism and physiology caused by drought stress can be reduced by selenium (Se) supplementation. Se increases the activity of antioxidant enzymes and reduces both the generation of reactive oxygen species (ROS) and the levels of lipid peroxidation (Habibi, 2013; Mostofa et al., 2017; Nawaz et al., 2015).

An increase in CO_2 assimilation rate and stomatal conductance is promoted by application of Se in wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) plants exposed to drought stress (Habibi, 2013; Nawaz et al., 2015). Similarly, in olive (*Olea europaea* L.) under water stress, foliar applications of Se can increase photosynthesis, regulate the water status of trees and maintain a sufficiently high level of leaf water content in plants (Proietti et al., 2013). Yao et al. (2009) verified significant increases in root activity, catalase activity and chlorophyll content as well as reduced malondialdehyde content in wheat seedlings subjected to Se application under drought stress.

Therefore, studies that demonstrate the positive effects of Se application on rice plants under water deficit conditions are highly relevant. For this reason, this study aimed to validate the effects of Se doses applied to the soil under two soil water conditions on the growth, productivity, antioxidant system and nutritional quality status of rice plants.

2. Materials and Methods

2.1. Experimental design and rice cultivation

The experiment was conducted in a greenhouse from July to December 2014 at the Soil Science Department of Federal University of Lavras, Brazil. Pots with 4 kg of Oxisol (clay textured) collected from the 0 – 0.20-m layer were used. The soil was air-dried, sieved using a 4-mm mesh and characterized for its major chemical and physical properties according to the methodology suggested by Embrapa (1997). The chemical and physical properties as follow: pH-H_2O = 5.1; organic matter = 46 g kg^{-1}; P (Mehlich-1) = 0.84 mg kg^{-1}; K = 1.7 mmolc dm^{-3}; Ca = 1.5 cmolc dm^{-3}; Mg = 0.4 cmolc dm^{-3}; Al = 0.5 cmolc dm^{-3}; H+Al = 6.3 cmolc dm^{-3}; P-rem = 11.37 mg L^{-1}; clay = 710 g kg^{-1}; silt = 140 g kg^{-1}; and sand = 150 g kg^{-1}. The content of Se was 0.2 mg kg^{-1}, which was determined after soil digestion according the USEPA 3051A method (USEPA, 1998).

Based on soil chemical analysis, liming was carried out to raise the base saturation to 60% using $CaCO_3$ and $MgCO_3$ at a ratio of 4:1. After 30 days of

soil incubation with a humidity close to 60% of the total pore volume (TPV), doses of Se (sodium selenate, Sigma-Aldrich, Saint Louis, USA) were applied to the soil. Each pot received a macronutrient fertilizer containing: 80 mg of N, 250 mg of P, 90 mg of K, and 50 mg of S kg^{-1} soil. Micronutrient applications consisted of 0.5 mg of B, 1.5 mg of Cu, 0.1 mg of Mo and 5.0 mg of Zn kg^{-1} soil.

Afterward, 15 rice seeds (*O. sativa* L. cv. IAC 202) were sown per pot, and nine days after seedling emergence, the rice seedlings were thinned three plants per pot. In addition, during the rice cultivation period, blanket fertilizations of 473 mg of N and 436 mg of K kg^{-1} soil were carried out and divided into five applications.

The experiments were set up as a completely randomized design in a 4 × 2 factorial scheme with four Se doses (0, 0.5, 1.0 and 2.0 mg kg^{-1} Se) and two soil water conditions (irrigated and water deficit), with four replicates, during the beginning of the reproductive stage. The control treatment consisted of maintaining the soil near field capacity, and water deficit treatment involved maintaining the soil at approximately -50 kPa, as determined by Stone et al. (1986) for rice cultivation. Each experimental unit consisted of two pots: one was used for the analyses performed at the end of water deficit, and the other was used at grain harvest.

To monitor soil water tension, tensiometers were installed at a 0.15-m depth in each experimental plot, and the replenishment of evapotranspired water

was performed based on the tensiometric reading, which was performed twice daily at 9:00 and 16:00 h. Appropriate water replenishment was used to determine water retention curves for the soil. From emergence to the flag leaf/collar-formation stage, field capacity was maintained by soil moisture in all pots.

In the flag leaf/collar-formation stage, water deficit treatments were applied to the respective pots, and soil water potential was maintained at -50 kPa for 14 days to simulate dry spells; samples not subjected to water deficit were maintained under normal irrigation conditions. At the end of this period, irrigation to plants under water stress was restored to field capacity until harvest.

2.2. Gas exchange measurements

On the 14th day of water stress at the end of water deficit period, which coincided with the initial panicle exsertion stage of rice, gas exchange evaluations were performed using a portable infrared gas analyzer (Infra Red Gas Analyzer - IRGA, brand LI-COR Biosciences, model LICOR 6400). The CO_2 assimilation rate expressed by area (*A* - μmol CO_2 m^{-2} s^{-1}), stomatal conductance (*gs* - mol H_2O m^{-2} s^{-1}), transpiration (*E* - mmol H_2O m^{-2} s^{-1}) and internal CO_2 concentration in the substomatal chamber (*Ci* - μmol CO_2 mol air^{-1}) were obtained. With those data, an estimation of both instantaneous carboxylation efficiency [*EiC*, (*A*/*Ci* - mol air^{-1})] and water use efficiency [*WUE*, (*A*/*E* - μmol CO_2 $mmol^{-1}$ H_2O)] were determined. Readings were performed on a

clear day between 9:00 and 11:00 am using the flag leaf as a pattern, including the last fully developed leaf. The photosynthetically active radiation (PAR) was standardized to an artificial saturating light of 1,000 µmol m^{-2} s^{-1} and an ambient CO_2 concentration. The average relative humidity was 30%, and the ambient temperature was between 35 to 42 °C.

2.3. Biometric and chlorophyll meter measurements

Using three leaves per pot on the same day, the SPAD index was determined using a chlorophyll meter (SPAD-502, Konica-Minolta, Japan). After SPAD readings, leaves were collected and immediately conditioned in liquid nitrogen (LN_2), after which they were stored at -80 °C for biochemical analyses. After all these determinations at end of the water deficit period, the height of plants at that time was obtained by measuring from the plant base to the end of last fully expanded leaf. The shoots and roots of plants were collected and dried in a forced-air oven for 72 hours in order to obtain and determine the dry biomass and root/shoot relation. The other post treatments aimed at grain production were conducted until the cycle ended, after which the grains were harvested, dried in a forced-air oven, and weighed to obtain the dry mass of grains in each pot.

2.4. Extraction and quantification of antioxidant enzymes

To quantify antioxidant enzyme activity in leaves, an extract was obtained by maceration of 0.1 g of leaves in LN_2. The extract was then added to an extraction buffer solution containing 0.1 M potassium phosphate (pH 7.8), 0.1 mM EDTA (pH 7.0), 0.01 M ascorbic acid and 22 mg of polyvinylpyrrolidone (PVPP) (Biemelt et al., 1998). The supernatant was then collected and used for enzymatic analyses of superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX).

2.4.1. Superoxide dismutase (SOD; EC 1.15.1.1)

SOD activity was evaluated by the ability of the enzyme to inhibit the photoreduction of nitroblue tetrazolium (NBT), as proposed by Giannopolitis and Ries (1977). An aliquot of the supernatant was added to an incubation medium composed of 50 mM potassium phosphate (pH 7.8), 14 mM methionine, 0.1 μM EDTA, 75 μM NBT and 2 μM riboflavin. Tubes with reaction media and samples were illuminated for 7 minutes with a 20-W fluorescent lamp. For the control, the same reaction medium was illuminated without the sample present. Readings were performed at 560 nm. One unit of SOD corresponds to the amount of enzyme capable of inhibiting the photoreduction of NBT by 50% under assay conditions.

2.4.2. Catalase (CAT; EC 1.11.1.6)

CAT was evaluated according to method described by Havir and McHale (1987), in which an aliquot of enzyme extract was added to an incubation medium containing 100 mM potassium phosphate (pH 7.0) that was previously incubated at 30 °C. Immediately prior to absorbance readings, 12.5 mM hydrogen peroxide was added to trigger this reaction. CAT activity was determined by measuring the consumption of hydrogen peroxide (H_2O_2) at 240 nm for 3 minutes. A molar extinction coefficient of 36 mM^{-1} cm^{-1} was used for calculations.

2.4.3. Ascorbate peroxidase (APX, EC 1.11.1.11)

APX activity was determined by monitoring the oxidation rate of ascorbate at 290 nm for 3 minutes. For this measurement, an aliquot of enzyme extract was added to an incubation buffer composed of 100 mM potassium phosphate (pH 7.0) and 0.5 mM ascorbic acid, which was previously incubated at 30 °C. Prior to measuring the oxidation rate, 0.1 mM H_2O_2 (Nakano and Asada, 1981) was added to the sample. A molar extinction coefficient of 2.8 mM^{-1} cm^{-1} was used for calculations.

2.5. Hydrogen peroxide

Hydrogen peroxide was quantified from an extract made by macerating 0.2 g of leaves in LN_2. The extract was then homogenized in 1.5 mL of trichloroacetic acid (TCA) and centrifuged at 12,000 g for 15 minutes at 4 °C.

Hydrogen peroxide levels were determined by measuring absorbance at 390 nm. Aliquots of the supernatant were added to a reaction medium containing 2.5 mM potassium phosphate buffer (pH 7.0) and 500 mM potassium iodide (Velikova et al., 2000). Quantification of H_2O_2 was performed based on a standard curve with known concentrations of H_2O_2.

2.6. Lipid peroxidation

Lipid peroxidation was determined by the quantification of thiobarbituric acid reactive species (TBA), as described by Buege and Aust (1978). A total of 0.2 g of leaves was macerated in LN_2 and 20% PVPP (w/v), which was homogenized in 0.1% (w/v) TCA. The homogenate was then centrifuged at 10,000 g for 10 minutes. Aliquots of the supernatant were added to a reaction medium containing 0.5% (w/v) TBA and 10% (w/v) TCA, after which the samples were incubated at 95 °C for 30 minutes. The reaction was stopped by cooling the samples on ice. Absorbance was measured using a spectrophotometer at 535 nm and 600 nm, and the results were expressed in nanomoles of malondialdehyde (MDA) per milligram of fresh weight (FW).

2.7. Chemical analysis of plant tissue

Shoot dry biomass and grains were ground in a Willey mill equipped with a 40-mesh screen. Subsequently, in the aerial parts of plants, chemical analyses were performed to determine macro and micronutrient contents in accordance

with the methodology described by Malavolta et al. (1997). To analyze Se levels in these tissues, 0.5 g of tissue was used for perchloric-nitric digestion. Extracts sat for 15 minutes, after which 10 mL of deionized water was added. For analytical quality control, analyses were certified using the BCR 402 (white clover) standard (Sigma-Aldrich). Blank and certified reference samples were analyzed along with the digestion of every batch, which presented a minimum of 90% (n = 7, SE = 6.27) recovery. The extracts were measured using atomic absorption spectroscopy with electrothermal atomization in a graphite furnace.

2.8. Statistical analysis

When all data sets were considered, normality was analyzed using the Anderson-Darling test, and homoscedasticity was verified using the variance equation test (or the Levene test). The data obtained for each variable were subjected to the analysis of variance ($p \leq 0.05$), and the factors were compared using the Scott-Knott test in R 3.2.3 statistical program (RDCT, 2011).

3. Results

3.1. Gas exchange and antioxidant metabolism

Interactions ($p \leq 0.05$) between soil water conditions and Se doses applied to *A*, *gs*, *E*, *EiC* and *WUE* (Figure 1 A, B, C, E and F) were observed. Internal CO_2 concentration (Table 1 and Figure 1 D), lipid peroxidation (Table 1), SOD activity (Figure 2 A) and H_2O_2 content (Table 1 and Figure 2 B) were

affected ($p \leq 0.05$) when analyzed individually by water condition and Se dose. APX and CAT were not affected by soil water condition or by Se dose.

Water deficit promoted the reduction of *A*, *gs*, *E* and *EiC* (Figure 1 A, B C and E) by all Se doses applied, except when *gs* were checked without Se application. For *WUE* estimation (Figure 1 F), the highest dose of Se (2.0 mg kg^{-1}) enabled an increase of this variable when plants were subjected to water deficit, and these results differed from those of irrigated plants. The *Ci* increased by 16.52% in plants under water deficit (Table 1).

Application of 1.0 mg kg^{-1} Se to irrigated plants usually promoted the highest values of *A*, *gs*, *E* and *EiC* (Figure 1 A, B, C and E), unlike plants that did not receive Se. For *WUE* (Figure 1 F), there were no differences among Se doses in irrigated plants. However, at the highest dose (2.0 mg kg^{-1}), there was reduction in *A*, *gs*, and *EiC*. In plants subjected to water deficit, application of Se promoted an increase in *A*, *E*, *EiC* and *WUE* (Figure 1 A, C, E and F) compared to plants that did not receive Se. The *gs* in plants subjected to water deficit did not vary with the dose of Se applied (Figure 1 B).

The *A* and *E* of plants under water deficit that received a dose of 1.0 mg kg^{-1} Se in the soil were 76.0 and 46.2% higher than those of plants that did not receive Se (Figure 1 A and C). The *Ci* decreased accordingly with increasing dose of Se applied, regardless of whether plants were under water stress or not (Figure 1 D). A dose of 0.5 mg kg^{-1} Se promoted a *Ci* that was approximately

15% lower than that of plants that did not receive Se application, although the results were not different compared to those of other doses.

Unlike the results for *Ci*, water deficit increased the concentrations of H_2O_2 and MDA by 19.4 and 28% in rice plants (Table 1), respectively. Application of 0.5 mg kg^{-1} Se promoted an increase in SOD activity and a decrease in H_2O_2 concentration (Figure 2 A and B) compared to those of plants that did not receive selenium regardless of the water regimen to which the rice plants were subjected.

3.2. Biometric measurements, chlorophyll readings and nutrient accumulations

Interactions ($p \leq 0.05$) between the water conditions of the soil and doses of Se regarding SPAD index and the accumulation of sulfur (S) (Figure 3 A and C) were observed. Plant height and copper (Cu) accumulation (Table 2 and Figure 3 B and D) were affected ($p \leq 0.05$) independently by water conditions and Se dose. Dry shoot, root, overall biomass and grain biomass as well as the accumulations of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), boron (B), iron (Fe), manganese (Mn) and zinc (Zn) (Table 2) were affected by soil water conditions. The root/shoot ratio was not affected by soil water conditions or Se dose.

The SPAD index was lower in plants cultivated under water deficit in the absence of Se and with application 1.0 mg kg^{-1} (Figure 3 A) compared to that of plants grown under normal water conditions. The highest SPAD index in

irrigated plants was recorded at the Se dose of 1.0 mg kg^{-1}, although no difference was observed in plants that did not receive Se. When comparing Se doses in the treatments of water stress, plants that received a 0.5 mg kg^{-1} Se dose had 8% higher SPAD index than in control plants.

The accumulation of S in plants under water stress was similar to S accumulation in irrigated plants that received 1.0 and 2.0 mg kg^{-1} Se (Figure 3 C). Increasing doses of Se did not cause significant accumulations of S in plants under the normal water regimen. In plants subjected to water deficit, there was significant increase in S accumulation in rice biomass (Figure 3 C).

Plant height increased with Se dose, regardless of water conditions. The 0.5 mg kg^{-1} dose of Se the provided the highest height, which was a 6.6% increase compared to that of untreated plants (Figure 3 B). The accumulation of Cu in rice plants increased linearly with increasing dose of Se applied to the soil (Figure 3 D), although no differences were observed between the doses of 0 and 0.5 mg kg^{-1} Se. The dose of 2.0 mg kg^{-1} Se increased Cu accumulation in the shoot biomass by 17.5% compared to that of plants that did not receive Se application.

Water deficit in rice plants caused reductions of 41%, 29.6%, 36.1%, 58.5% and 10.3% in shoot dry biomass, root dry biomass, overall dry biomass, grain production and plant height, respectively. Similarly, the accumulations of

N, P, K, Ca, Mg, B, Cu, Fe, Mn and Zn decreased under water deficit conditions in rice plants (Table 2).

3.3. Se accumulation and concentration in grain

Se accumulation in shoots (Figure 4 A) was affected ($p \leq 0.05$) only by the applied dose of Se. For Se content in grains, interactions ($p \leq 0.05$) between soil water conditions and Se dose (Figure 4 B) were observed. The accumulation of Se in the biomass of rice plants increased linearly with increasing application of Se dose regardless of water regimen (Figure 4 A). The highest accumulation of Se in the biomass (2.10 mg pot^{-1}) was recorded in the treatment consisting of the highest dose of Se. Water deficit promoted a reduction in the levels of Se in the grains of plants that received doses of 1.0 and 2.0 mg kg^{-1} Se. Se content in rice grains increased linearly from 0 to 20.78 mg kg^{-1} under water deficit conditions and from 0 to 23.58 mg kg^{-1} under irrigated conditions (Figure 4 B).

4. Discussion

Our results demonstrated that water stress reduces photosynthesis, growth, nutrient absorption and productivity. However, Se application increased *A*, *gs*, *E* and *WUE* in plants under water stress. The reductions of *A*, *gs*, *E* and *EiC* (Figure 1 A, B, C and E) in plants subjected to water deficit at all doses of Se are related to the negative effects of water restriction on these variables. On the other hand, an increase in *WUE* at the dose of 2.0 mg kg^{-1} Se (Figure 1 F)

was also observed in these plants. Water deficit significantly reduces the growth of cultivated plants due to the direct influence of water deficit on various physiological and biochemical processes (Ahmad et al., 2016b).

Decreases in *A* and *gs* in plants experiencing water deficit may be due to chlorophyll degradation and fragmentation, the suppression of rubisco activity and stomatal closure (Jákli et al., 2017; Zhang et al., 2017). Transpiration is an essential process for plants during photosynthesis. A reduction in transpiration to maintain internal water balance when plants are subjected to water deficit, as described by Yan et al. (2016), was verified in this study. Zain et al. (2014) reported a decrease in *A*, *gs* and *E* as water deficit increased while subjecting rice plants to water deficit, which was also observed in this study.

Although water stress reduced *A, gs*, *E*, *EiC* and *WUE*, Se application attenuates these reductions, which was verified in our study. This is related to the protective Se effects, as this element minimizes damage to chloroplast structure and promotes greater activity of the electron transport system compared to the control. Nawaz et al. (2015) and Proietti et al. (2013) reported positive effects of Se application on *A*, *gs* and *E* in wheat and olive plants under both normal water and water deficit conditions.

The lowest *EiC* in plants subjected to water stress in the present study is associated with a limitation in CO_2 assimilation (Figure 1 A) caused by stomatal closure (Figure 1 B). Stomatal closure is considered the main cause of

decreasing rates of photosynthesis in C3 plants subjected to moderate water deficit (Flexas and Medrano, 2002). However, under more severe stress conditions, *Ci* increases, which indicates a predominance of non-stomatal limitations to photosynthesis, as reported by Mafakheri et al. (2010).

The increase in *WUE* with increasing Se dose in rice plants under water deficit is related to the activation of osmotically active compounds by this element. Nawaz et al. (2016) reported the accumulation of osmotically active molecules in corn (*Zea mays* L.) under conditions of water restriction associated with Se applications, which resulted in the greatest *WUE* by the plant.

The highest concentrations of H_2O_2 and lipid peroxidation (Table 1) in rice plants subjected to water deficit could be related to the disturbance between generation and extinction of reactive oxygen species. This is because water stress reduces the water content in plants, which leads to the degradation of pigments (Figure 3 A), reduction of the photochemical phase, reduction of CO_2 assimilation (Figure 1 A), breakage of homeostasis and elevation of ROS generation (Faize et al., 2011). Habibi (2013) and Zain et al. (2014) reported increases in the concentrations of lipid peroxidation and H_2O_2 in barley and rice, respectively, in plants subjected to water deficit.

An increase in SOD enzyme activity associated with decreased *Ci* and H_2O_2 in rice plants due to Se application in the present study shows the protective role of this element, as reported by Feng et al. (2012) and Yao et al.

(2013). Even though SOD is not a selenoenzyme, Se increases gene expression and activity levels of the SOD enzyme. Jiang et al. (2017) reported that genes involved in SOD activation mechanisms are significantly upregulated in maize roots 24 hours after applications of 1 μM Se. Nawaz et al. (2016) reported an increase in SOD activity in corn plants under normal irrigation conditions after the addition of Se to soil.

A reduction in H_2O_2 content is related to increased SOD activity (Figure 2 A) as a result of Se application, as SOD is the first line of antioxidative defense against ROS. An increase in SOD activity after Se application provides evidence that this element may be directly involved in the extinction of superoxide (O_2^-) and hydroxyl radicals (OH^-) in cells (Nawaz et al., 2016).

Water deficit reduced the height, biomass, and grain yield of rice plants, and these changes may be related to numerous processes in the plant in which water participates, with an emphasis on maintaining turgor and mitotic impairment (Jaleel et al., 2009). When water is reduced due to water deficit, turgescence severely reduces cell growth (Flexas, 2016). Water deficit affects both stretching and cell growth and inhibits cell growth rather than cell division (Jaleel et al., 2009; Shao et al., 2008).

A decrease in plant height subjected to water deficit is related to leaf primordia formation, which is a process that is sensitive to water restriction. The negative effects of water deficit on productivity are due to low shoot and root

biomass production, reduced soil nutrient utilization and reduced plant photosynthesis, which were both shown in this study and reported by Feng et al. (2012). A decrease in productivity, growth and biomass was reported by Zain et al. (2014) and Nawaz et al. (2015) in rice and wheat plants subjected to water restrictions.

Se has beneficial effects on the growth of plants, especially in plants exposed to stress conditions. Strong growth of plants that received Se is related to improvements promoted by photosynthesis (Figure 1) and by the reduced formation of reactive oxygen species, lipid peroxidation and formation of H_2O_2 in plant cells (Figure 2 B). Hashem et al. (2013) reported an increase in canola (*Brassica napus* L.) growth when the plants received foliar applications of 2.5 and 5.0 mg L^{-1} Se.

Drought stress is usually characterized by a reduction of chlorophyll and is associated with a progressive decline in the photosynthetic capacity of plants, which explains the lower SPAD values in plants experiencing water deficit compared to those of plants irrigated regularly. An increase of this index in plants under both water deficit conditions and Se treatment is associated with an increase in chlorophyll content, which is in agreement with the results of Iqbal et al. (2015). A decrease in SPAD index at the highest dose of Se is caused both by an adverse effect on porphobilinogen synthase production, which is necessary

for chlorophyll biosynthesis, and the inhibition of biosynthetic enzymes through lipid peroxidation (Saffaryazdi et al., 2012).

An increase in S biomass accumulation in plants that were subjected to water deficit and that received doses of Se may be related to synergistic interactions between selenate and sulfate ions due to their similar chemical properties and use of the same membrane transporter and route of assimilation (Pilon-Smits, 2015; White, 2016). An increase in S content in plants that received 1.0 g kg^{-1} Se under water deficit conditions may have occurred because the stress promoted an increase in glutathione synthesis due to the need to reduce reactive oxygen species (Table 1). Se has a direct effect on enzyme synthesis. Thus, the synthesis requirement of Se for reduced S may have led to the accumulation of this element (Ahmad et al., 2016a; Sajedi et al., 2011). This accumulation was equal to that in plants under a normal water regimen at those doses.

The increase in Cu accumulation in rice plants due to Se doses differed from the results reported by Schiavon et al. (2013), who detected no effect on Cu accumulation in the shoots of tomato (*Solanum lycopersicon* L.). Arvy et al. (1995) reported increases in Cu in the biomass of *Catharanthus roseus* (L.) plants as a result of Se treatment. Cu, Fe, Zn and Mn are important SOD cofactors (Yao et al., 2013) that may have caused an increase in plant growth because of the high activity of the SOD enzyme (Figure 2 A).

The accumulation of nutrients in the shoots of rice plants under water deficit conditions decreased because of the reduction in mobility and absorption of nutrients. Under water stress conditions, roots are unable to absorb many nutrients from the soil due to reduced root activity, which is associated with decreased water movement toward the roots and slower ion diffusion (Alam, 1999). Sardans and Peñuelas (2004) reported that a 22% reduction in soil moisture decreased the amount of P accumulated in plants by 40%; this reduction mainly occurred because there was no decrease in shoot biomass. In addition, Dejong and Phillips (1982) reported a decrease of 50% in the accumulation of N in clover (*Trifolium subterraneum* L. cv. Woogenellup).

An increase in Se levels in the shoots and grains of rice plants subjected to the application of this element was demonstrated by Hu et al. (2002), Boldrin et al. (2013), Hu et al. (2014) and Nothstein et al. (2016). This increase is related to the Se concentration mainly in the soil. The accumulation of Se in rice plant biomass is an important content predictor of this element in the grain, as indicated in the study by Zhang et al. (2006) and confirmed in this present study.

In addition, the intended outcome of biofortification programs is an increase in Se levels in plants and grains without harming productivity. In this regard, the doses applied in the present study did not promote a significant reduction in plant biomass or grains. The results showed photosynthesis improvements and increased water use efficiency in plants subjected to water

deficit, which demonstrates that Se is important for enhancing the development of plants under water deficit conditions.

Conclusion

Se application to rice plants improves their CO_2 assimilation rate, transpiration rate, instantaneous carboxylation efficiency, water use efficiency estimation, and physiological capacity to withstand water deficit. There was a decrease in H_2O_2, and increases in SOD activity, and accumulations of plant Se and grain content due to applied Se doses.

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Table 1. Effects of water conditions on internal CO_2 concentration (*Ci*), hydrogen peroxide (H_2O_2) and lipid peroxidation (MDA) in rice plants.

Water condition	*Ci* (μmol m^{-2} s^{-1})	H_2O_2 (μmol H_2O_2 mg^{-1} FW)	MDA (nmol mg^{-1} FW)
Irrigated	$179.03^{b} \pm 3.94$	$11.84^{b} \pm 0.63$	$82.45^{b} \pm 4.93$
Water deficit	$208.59^{a} \pm 7.09$	$14.69^{a} \pm 0.82$	$114.62^{a} \pm 4.22$

Means not sharing the same letter, a and b, differ significantly ($p < 0.05$) within each column. ± standard error of average values (n = 4).

Table 2. Growth, grain biomass and nutrient accumulation in rice plants under different soil water conditions.

Growth					
Water condition	Shoot dry biomass	Root dry biomass	Overall dry biomass	Height of plants	Grain biomass
	(g pot^{-1})	(g pot^{-1})	(g pot^{-1})	(cm)	(g pot^{-1})
Irrigated	79.25^{a} ±0.84	60.63^{a} ±2.72	139.88^{a} ±2.76	93.60^{a} ±0.84	75.26^{a} ±1.42
Water deficit	46.75^{b} ±0.52	42.63^{b} ±3.31	89.38^{b} ±3.40	83.93^{b} ±0.77	31.19^{b} ±1.60
Nutrient accumulation					
Water condition	N	P	K	Ca	Mg
	g pot^{-1}				
Irrigated	1.487^{a} ±0.031	0.127^{a} ±0.005	1.720^{a} ±0.044	0.194^{a} ±0.011	0.249^{a} ±0.011
Water deficit	1.136^{b} ±0.039	0.083^{b} ±0.003	1.125^{b} ±0.027	0.149^{b} ±0.008	0.171^{b} ±0.011
Water condition	B	Cu	Fe	Mn	Zn
	mg pot^{-1}				
Irrigated	1.097^{a} ±0.048	0.74^{a} ±0.021	9.81^{a} ±0.451	84.39^{a} ±4.82	3.48^{a} ±0.111
Water deficit	0.799^{b} ±0.044	0.42^{b} ±0.013	7.24^{b} ±0.326	59.95^{b} ±1.75	2.63^{b} ±0.122

Means not sharing the same letter, a and b, differ significantly ($p < 0.05$) within each column. ± standard error of average values (n = 4).

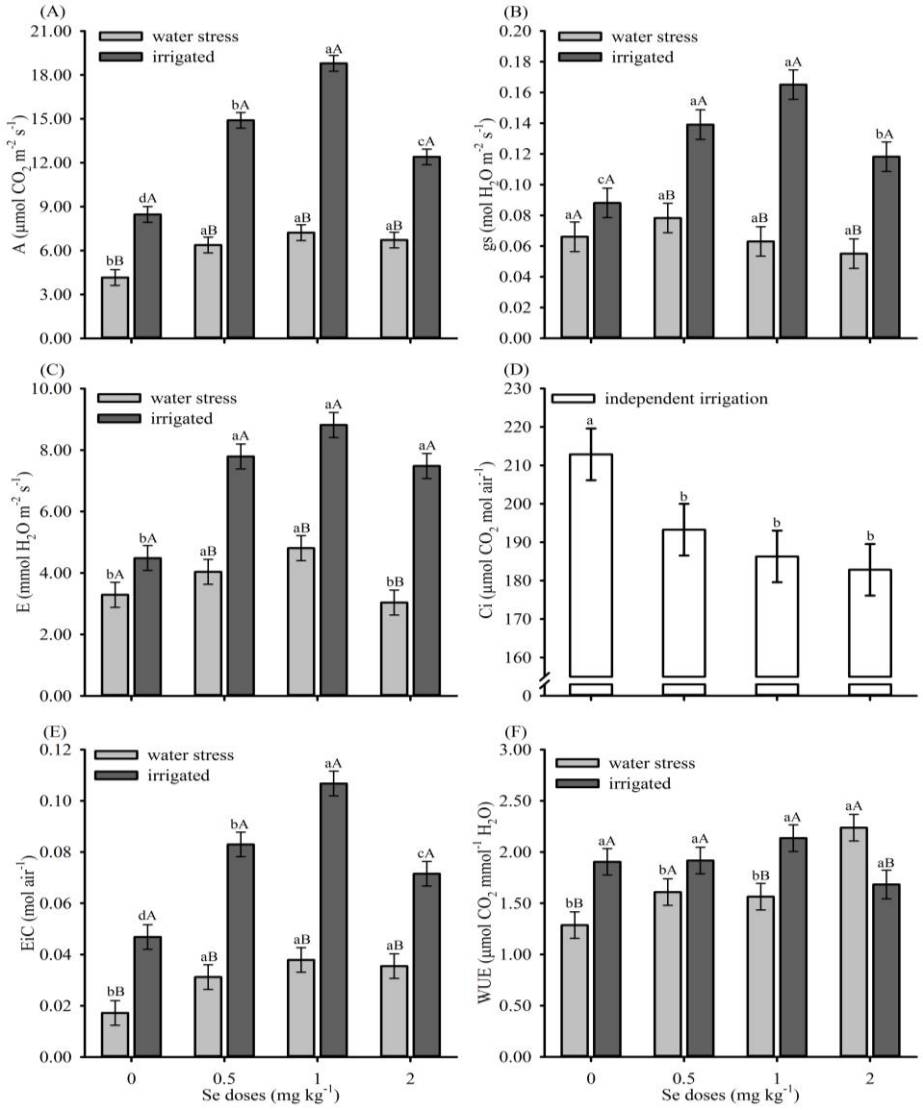

Fig. 1. Effects of soil water conditions and Se doses on the CO_2 assimilation rate (A), stomatal conductance (B), transpiration rate (C), internal CO_2 concentration (D), instantaneous efficiency of carboxylation (E) and water use efficiency (F) of rice plants. Uppercase letters compare the means soil water conditions in each Se dose, while lowercase letters compare the means of the Se doses at each soil water conditions; lowercase letters alone at bars, compare the means of the Se doses independent of soil water conditions, according to the Scott-Knott test (5% probability). The vertical bars indicate the standard errors of the average values (n = 4).

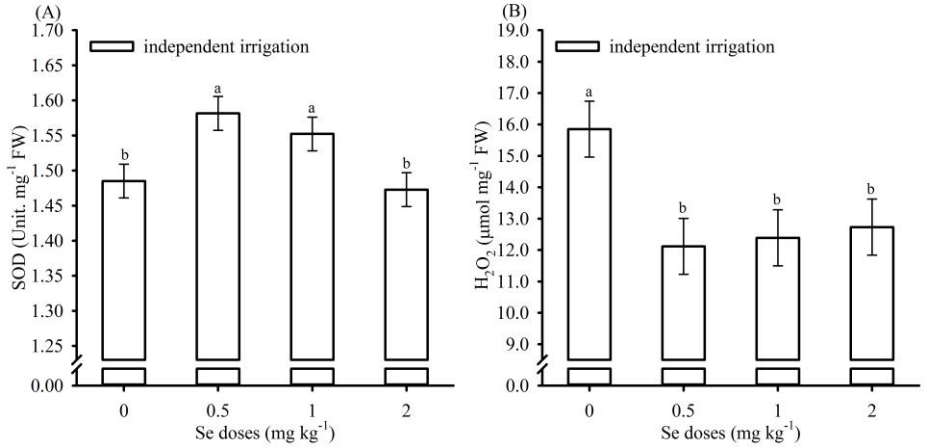

Fig. 2. Effect of Se dose on the activity of superoxide dismutase (A) and the concentration of hydrogen peroxide (B) in rice leaves. Means followed by same lowercase letter do not differ from each other according to the Scott-Knott test (5%). The vertical bars indicate the standard errors of the average values (n = 4).

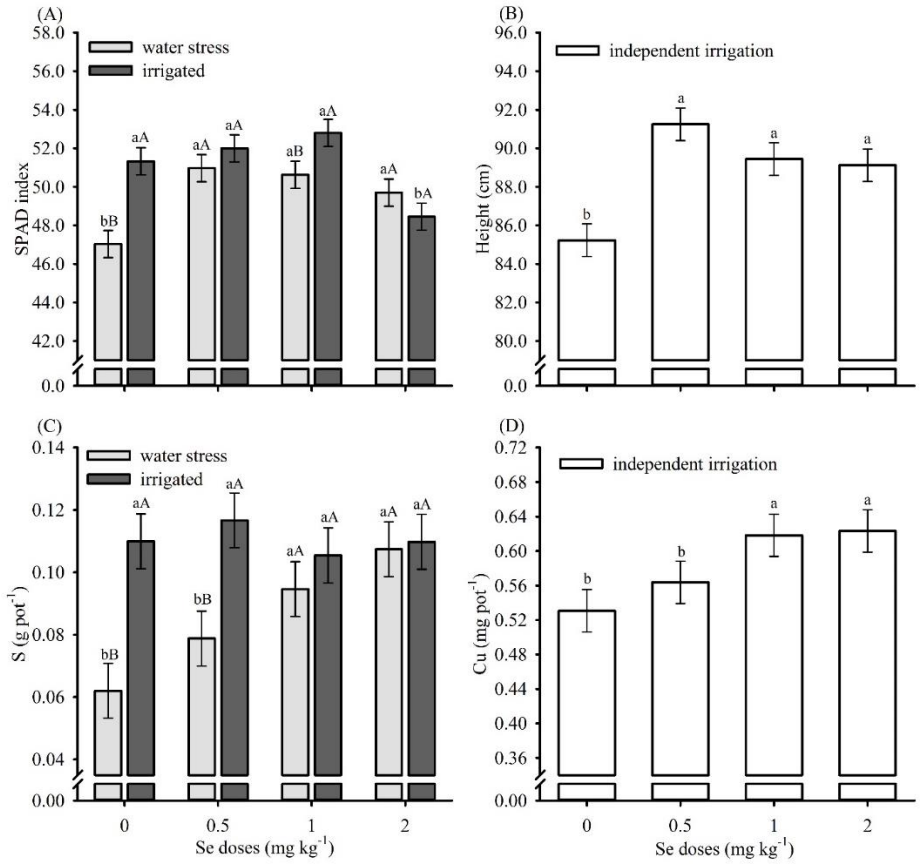

Fig. 3. Effects of soil water condition and Se dose on the SPAD index (A), height (B), and accumulations of S (C) and Cu (D) in rice plants. Uppercase letters compare the means soil water conditions in each Se dose, while lowercase letters compare the means of the Se doses at each soil water conditions; lowercase letters alone at bars, compare the means of the Se doses independent of soil water conditions, according to the Scott-Knott test (5% probability). The vertical bars indicate the standard errors of average values (n = 4).

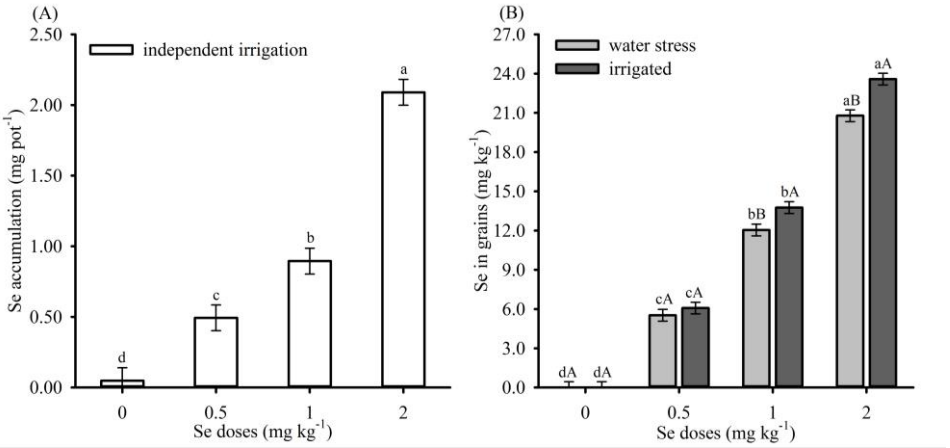

Fig. 4. Effects of soil water condition and Se dose on Se accumulation in shoot biomass (A) and Se grain content (B) in rice plants. Uppercase letters compare the means soil water conditions in each Se dose, while lowercase letters compare the means of the Se doses at each soil water conditions; lowercase letters alone at bars, compare the means of the Se doses independent of soil water conditions, according to the Scott-Knott test (5% probability). The vertical bars indicate the standard errors of average values (n = 4).