UNIVERSIDADE FEDERAL DE LAVRAS

RUBY ANTONIETA VEGA RAVELLO

SOIL SELENIUM (Se) APPLICATION FOR Se BIOFORTIFICATION AND FOR PROTECTING *Phaseolus vulgaris* L. AND *Chenopodium quinoa* Willd. AGAINST WATER DEFICIT

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> Doctoral dissertation 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 degree of Doctor in Soil Science.

Prof. Dr. Guilherme Lopes

Advisor

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This study is dedicated to Almighty God as my companion and the light guiding my path. To my beloved parents for their unconditional love and for being the support and engine of my life. Thank you, Mom, for teaching me never to give up and for lighting up my days with your sweet smile, and Dad, for never hesitating to hold my hand and walk by my side, giving me strength and confidence to move forward. To my sisters and brother, the four of us are the components of the same heart that beats because we always are joined; I love you and more for those seven beautiful "Pirañas."

Dedication

ACKNOWLEDGMENT

El tiempo de Dios es perfecto.

RESUMO GERAL

Alimentos deficientes em selênio afetam negativamente a saúde humana, visto que o Se é um elemento essencial para os processos biológicos dos seres humanos. A biofortificação de culturas tornou possível enriquecer alimentos com selênio cultivados em solos com baixos níveis de Se para garantir a ingestão adequada de Se na dieta humana. Além disso, o Se é um elemento benéfico para as plantas, mas seu papel metabólico essencial às plantas ainda não foi determinado. A ação mais proeminente do Se nas plantas é seu o mecanismo de proteção antioxidante, que promove a atividade de sistemas enzimáticos e não enzimáticos para lidar com o estresse abiótico, como o déficit hídrico. Nesse contexto, foram realizados três estudos em casa de vegetação para determinar o efeito da aplicação de Se no solo, no enriquecimento de grãos de feijão e quinoa com selênio e avaliar o efeito da aplicação de Se sobre os processos metabólicos e fisiológicos associados à tolerância ao déficit hídrico. Assim, a melhoria da qualidade nutricional dos grãos e o efeito de tolerância das plantas contribuiriam para o combate à fome oculta e à escassez de água, ameaças latentes à segurança alimentar. Para o presente estudo, o selenato de sódio foi utilizado como fonte de Se e foram avaliados parâmetros fisiológicos e metabólicos em plantas submetidas a duas condições hídricas do solo: irrigado e com déficit hídrico. O primeiro estudo foi instalado no Brasil com *Phaseolus vulgaris* L. cultivar BRS Estilo, e cinco doses de Se (0, 0,25, 0,5, 1,0 e 2,0 mg kg^{-1}) foram aplicadas a um LATOSSOLO VERMELHO-AMARELO Distrófico (pH = 4,6; matéria orgânica = 32,7 g kg^{-1}; argila = 610 g kg^{-1}). A biofortificação agronômica de selênio enriqueceu plantas de feijoeiro com Se, e a atividade de enzimas antioxidantes apresentou respostas positivas na proteção das plantas contra déficit hídrico quando utilizadas em menores doses de Se, principalmente em 0,25 e 0,5 mg kg^{-1}. O segundo estudo foi realizado no Peru com *Chenopodium quinoa* W. var INIA Salcedo, e cinco doses de Se (0, 0,25, 0,5, 1,0 e 2,0 mg kg^{-1}) foram aplicadas a um NEOSSOLO FLÚVICO Ta Eutrófico (pH = 8,09; matéria orgânica = 11,5 g kg^{-1}; argila = 320 g kg^{-1}). A biofortificação de selênio enriqueceu os grãos de quinoa com selênio; enquanto isso, a atividade antioxidante não enzimática pode ser um dos mecanismos de tolerância ao déficit hídrico promovido pelo Se na quinoa var INIA Salcedo potencializada principalmente na dose 0,5 mg kg^{-1} de Se. O último estudo foi realizado com *Chenopodium quinoa* W. cultivar BRS-Piabiru, e sete doses de Se (0, 0,125, 0,25, 0,5, 0,75, 1,0 e 2,0 mg kg^{-1}) foram aplicadas a um LATOSSOLO VERMELHO-AMARELO Distrófico (pH = 4,6; matéria orgânica = 32,7 g kg^{-1}; argila = 610 g kg^{-1}). A biofortificação de selênio via solo melhorou a qualidade nutricional dos grãos de quinoa com selênio e a atividade de enzimas antioxidantes. O selênio também promoveu acúmulo de aminoácidos livres, o que por sua vez aumentou a tolerância ao déficit hídrico. A ação integrada desses mecanismos de defesa mitigou o estresse oxidativo severo, regulando as trocas gasosas que permitiram a continuidade do processo fotossintético da quinoa cultivar BRS-Piabiru, principalmente na dose de 0,75 mg kg^{-1}.

Palavras-chave: Estresse abiótico. Sistema de defesa das plantas. Tolerância vegetal. Atividade antioxidante enzimática. Atividade antioxidante não enzimática.

GENERAL ABSTRACT

Selenium-deficient food negatively affects human health, as Se is an essential element for human biological processes. Crop biofortification has made it possible to enrich cultivated plants with selenium in soils containing low Se levels to ensure a proper Se intake in the human diet. Selenium is a beneficial element for plants but its essential metabolic role in plants has not yet been determined. The most prominent action of Se in plants is its role in the antioxidant protection mechanism, which promotes the activity of enzymatic and non-enzymatic systems to cope with abiotic stresses, such as water deficit. In this context, three greenhouse studies were performed to determine the effect of soil Se application on the enrichment of bean and quinoa grains with selenium and to evaluate the effect of applying Se on metabolic and physiological processes that are associated with tolerance to water deficit. By improving the nutritional quality of grains, while also increasing plant tolerance to water deficits we could contribute to minimize problems related to hidden hunger and water shortage, which are latent threats to food security. For the present study, sodium selenate was used as the source of Se and we evaluated physiological and metabolic parameters in plants subjected to two soil water conditions: irrigated and water deficit. The first study was installed in Brazil with *Phaseolus vulgaris* L. cultivar BRS Estilo, and five Se rates (0, 0.25, 0.5, 1.0, and 2.0 mg kg^{-1}) were applied to an Oxisol (pH = 4.6; SOM = 32.7 g kg^{-1}; clay = 610 g kg^{-1}). Agronomic selenium biofortification enriched common bean plants with Se, and antioxidant enzyme activity showed positive responses to plant protection against water deficit when used at lower Se rates, mainly at 0.25 and 0.5 mg kg^{-1}. The second study was conducted in Peru with *Chenopodium quinoa* W. var INIA Salcedo, and five Se rates (0, 0.25, 0.5, 1.0, and 2.0 mg kg^{-1}) were applied to an Entisol (pH = 8.09; SOM = 11.5 g kg^{-1}; clay = 320 g kg^{-1}). Selenium biofortification enriched quinoa grains with selenium; meanwhile, antioxidant non-enzymatic activity might be one of the water deficit tolerance mechanisms promoted by Se on quinoa var INIA Salcedo and would be enhanced mainly at 0.5 mg kg^{-1} Se. The last study was carried out with *Chenopodium quinoa* W. cultivar BRS-Piabiru, and seven Se rates (0, 0.125, 0.25, 0.5, 0.75, 1.0, and 2.0 mg kg^{-1}) were applied to an Oxisol (pH = 4.6; SOM = 32.7 g kg^{-1}; clay = 610 g kg^{-1}). Soil selenium biofortification enhanced quinoa grains' nutritional quality with selenium and antioxidant enzyme activity. Selenium also promoted free amino acid accumulation, which in turn enhanced the tolerance to water deficit. The integrated action of these defense mechanisms mitigated severe oxidative stress, thus regulating gas exchange that allowed the continuity of the photosynthetic process of quinoa cultivar BRS-Piabiru, particularly at 0.75 mg kg^{-1}.

Keywords: Abiotic stress. Plants' defense system. Plant tolerance. Enzymatic antioxidant activity. Non-enzymatic antioxidant activity.

CONTENTS

FIRST PART

GENERAL INTRODUCTION

The world population was estimated at 7 875 million in 2021, of which 9.8 percent face hunger, and 17.6 and 11.7 percent are affected by moderately and severely food insecurity, respectively (FAO et al., 2022). In addition, more than 2 billion people around the world have a micronutrient-deficient diet or malnutrition condition, which is known as "hidden hunger" (FAO; WFP; IFAD, 2012), and one billion people have a low Se dietary intake (LIMA; PILON-SMITS; SCHIAVON, 2018; SCHIAVON et al., 2020). Although the information available represents an estimated of the actual data and only considers nutritional deficiency in certain nutrients such as iron (Fe), zinc (Zn), iodine (I), and vitamin A, it is still a valuable reference for raising awareness that the world's population intakes suboptimal diets, and it must be addressed with the right policies to reduce malnutrition (SCHIBBA et al., 2020). In this regard, biofortification represents a sustainable agricultural strategy to address "hidden hunger" (DHALIWAL et al., 2022; HARVESTPLUS; FAO, 2019; LANZA; REIS, 2021; LOWE, 2021) by increasing vitamins and minerals in crops, principally Fe, Zn, I (BOUIS; SALTZMAN; BIROL, 2019; DHALIWAL et al., 2022), and selenium (Se) (DAS et al., 2017; LOPES; ÁVILA; GUILHERME, 2017). Thus, biofortification is a powerful method for nutrient enhancement of staple foods and bringing healthy diets because it provides humans with diversity and balanced content of macronutrients (proteins, fats, carbohydrates, and fiber) and essential micronutrients (vitamins and minerals) (FAO et al., 2021); biofortified staple food crops are enriched and provide bioavailable micronutrients (HARVESTPLUS; FAO, 2019). Biofortification strategies used to enrich crops with bioavailable micronutrients include agronomic practices, conventional plant breeding, or modern biotechnology (DAS et al., 2017; DHALIWAL et al., 2022; GARCIA-CASAL; PEÑA-ROSAS; GIYOSE, 2017). At agronomic biofortification, micronutrients are applied to the soil at planting and/or through plant development to enhance their micronutrient contents in edible crop parts. Soil application supplies enough amounts of nutrients for root uptake, whereas foliar application increases the nutrient content in leaves for redistribution in the plant. Therefore, biofortification efficiency relies on fertilization application mode, soil properties, and used amendments, since they affect crop enrichment and availability of micronutrients (DHALIWAL et al., 2022). Also, in recent years, these strategies have been considered promissory tools in biofortifying crops such as wheat, sweet potato, beans, rice, corn, wheat, potato, pearl millet, cassava, banana, cowpea, sorghum with essential micronutrients (BOUIS; SALTZMAN; BIROL, 2019; DHALIWAL et al., 2022; HARVESTPLUS; FAO, 2019).

Selenium was discovered by Jacob Berzelius, a Swedish chemist, in 1817 (FORDYCE, 2007; HOSSAIN et al., 2021; KIELISZEK, 2019), and its discovery triggered a large of innovative scientific research on human health (EL-RAMADY et al., 2020) because Se was known to be an essential element for humans (JAMES E. OLDFIELD, 2002). This element belongs to group 16 of the periodic table, called oxoacids. Also, Se is present in the environment in oxidation states from −2 to +6: selenides (Se^{2-}), elemental state (Se^0), selenites (SeO_3^{2-}), and selenates (SeO_4^{2-}), and these transformations depend on factors such as pH, the concentration of free oxygen, redox potential, and humidity (FORDYCE, 2007; KIELISZEK, 2019). Selenides constitute metal selenide (insoluble), volatile compound hydrogen gas selenide (H_2Se), and the organic forms of Se (solubles), such as selenocysteine (SeCys) "the 21st amino acid" (D'AMATO et al., 2020; WINKEL et al., 2015), which is convert to selenomethionine (SeMet) (EL-RAMADY et al., 2020; KIELISZEK, 2019; LIMA; PILON-SMITS; SCHIAVON, 2018). Selenium is the only micronutrient that is incorporated at the catalytic center of specific proteins, called "selenoproteins" (LIMA; PILON-SMITS; SCHIAVON, 2018; SCHIAVON et al., 2020). On the other hand, lower and higher oxidation states predominate under anaerobic conditions and acidic environments, and aerobic conditions and at alkaline pH, respectively (KIELISZEK, 2019).

Selenium is a micronutrient that plays an essential role in the functioning of biological processes for humans and animals. This element integrates the active center of selenoproteins, such as glutathione peroxidase antioxidant enzyme that catalyzes the biosynthesis of glutathione to mitigate oxidative damage of free radicals and/or regulation of intracellular redox state of the human brain and cardiovascular, immune, endocrine, and reproductive systems (D'AMATO et al., 2020; EL-RAMADY et al., 2020; HOSSAIN et al., 2021; KIELISZEK, 2019; PILON-SMITS, 2019; SARWAR et al., 2020; SCHIAVON et al., 2020; WINKEL et al., 2015). However, the narrow range between the deficiency and toxicity of Se represents a double-edged sword, which is why it is called "the essential poison" (EL-RAMADY et al., 2020; LIMA; PILON-SMITS; SCHIAVON, 2018). Therefore, the World Health Organization (WHO) recommends a daily Se intake of 55 μg for adults. Likewise, the Food and Nutrition Board (FNB) in the US considers that the Se intake varies with age and accounts for a range of 40–70 for men, 45–55 for women, and 25 μg for children (KIELISZEK, 2019). Nevertheless, the Se amount obtained from the diet is diverse and depends on crop Se content. Several plant-based foods worldwide contain insufficient Se levels for the human diet since they are cultivated in selenium-deficient soils, which increases the risk of human health disorders (DAS et al., 2017; DOS REIS et al., 2017; JAMES E. OLDFIELD, 2002; LANZA; REIS, 2021; RAINA et

al., 2021). There is a close interrelationship between the Se status of soil, crops, and humans. For instance, the Se intake of humans depends primarily on the Se content of soils and thus on the Se content of food crops grown on these soils (DOS REIS et al., 2017). Soils are very heterogeneous in their Se contents, most of which are low Se levels ranging from 0.01 to 2 mg kg^{-1} (LIMA; PILON-SMITS; SCHIAVON, 2018; RAINA et al., 2021; SCHIAVON et al., 2020). Therefore, Se-deficient soil represents an emerging issue for human health, where agronomic biofortification with Se in plants is an excellent alternative to combat the occurrence of low Se intakes in humans worldwide (DOS REIS et al., 2017; SARWAR et al., 2020) since Se inorganic uptake by the plant is converted into Se organic, which is more bioavailable (D'AMATO et al., 2020; HOSSAIN et al., 2021; KIELISZEK, 2019; |LIMA; PILON-SMITS; SCHIAVON, 2018). Studies report that foliar application of selenium is safer, more effective, easier, and more cost-efficient; however, its used low rates do not allow it to manifest its beneficial action on plants that are subjected to stresses such as water deficit (HOSSAIN et al., 2021; LANZA; REIS, 2021; SARWAR et al., 2020). Besides, the success of Se biofortification largely depends on understanding the mechanisms of Se uptake, assimilation, and tolerance by plants (HOSSAIN et al., 2021; LIMA; PILON-SMITS; SCHIAVON, 2018).

On the other hand, even though selenium does not perform an essential function for higher plants, this exhibits a beneficial role (BROWN; ZHAO; DOBERMANN, 2021) by increasing plant tolerance against stresses, which involves mechanisms that promotes the plant's defense system through enzymatic and non-enzymatic antioxidant activity. Uptake and accumulation of Se by plants include Se in the food chain; however, spite no specific uptake and transport mechanism in plants, Se enters by roots as selenate through sulfate transporters due to its physicochemical similarity with sulfur (S). The participation of Se in mechanisms of defense includes being an enzymatic cofactor as part of the catalytic center of selenoproteins, scavenging reactive oxygen species (ROS), and enhancing the antioxidant activity of enzymes such as superoxide dismutase (SOD) as the first line of defense, and catalase (CAT) and ascorbate peroxidase (APX) with complementary antioxidant activities. Also, accumulate compatible solutes such as proline and free amino acids to protect enzymes and proteins that can suffer degradation, especially in photosynthesis which is susceptible to being disturbed by dynamic enzyme activity and structural proteins in the organelles involved in this process (CHAUHAN et al., 2019; GUPTA; GUPTA, 2017; HASANUZZAMAN et al., 2020; LANZA; REIS, 2021; PILON-SMITS, 2019; SARWAR et al., 2020), such as chlorophyll pigment-protein complex (FAROOQ et al., 2012). As a result of this beneficial role, gas exchange enhances since stomatal conductance, transpiration rate, and CO$_2$ assimilation rate increase and

trigger the improvement of instantaneous carboxylation efficiency because chloroplast membranes are protected and the photosynthetic process is regulated to continue and ensure crop growth, and yield and grains quality under water deficit (FLEXAS et al., 2012; LANZA; REIS, 2021; SARWAR et al., 2020). This favorable effect of Se supports its use where environmental factors threaten food security, particularly staple food, which is essential to address since climate change intensifies water scarcity in agricultural systems (UNITED NATIONS, 2021) and disturbs morphological, physiological, and biochemical processes of crops at different degrees depending on the species and their phenological stages (FAROOQ et al., 2012; FAROOQI et al., 2020; FLEXAS et al., 2012), causing the reduction of crop production (UNESCO, 2020).

Staple foods are a predominant part of the human diet; their daily intake provides energy and covers nutritional needs; however, their consumption depends on the region's geographical features (DHALIWAL et al., 2022). Common bean (*Phaseolus vulgaris* L.) is the most important legume cultivated around the world since its grains contain high levels of protein, carbohydrates, vitamins, and minerals. Common bean is a staple food in the Brazilian diet; thus, Brazil is one of the biggest producers, harvesting above 3 million tons from 2.7 million ha in 2020 (CONAB, 2020; FAOSTAT, 2022). Quinoa (*Chenopodium quinoa* W.) is a pseudo-cereal domesticated by ancestral Andean people and is denominated as the "golden grain" (ANGELI et al., 2020) because its grains contain an exceptional balance of essential amino acids. This feature makes quinoa grains a food with higher quantity and protein quality than cereals, and also their level of lipids, proteins, dietary fibers, vitamins, and minerals are higher. Besides, it has wide genetic diversity that allows it to adapt to marginal conditions (FAO/RLC, 2011; FILHO et al., 2017; JACOBSEN, 2003). To date, Peru is the world's leading quinoa producer, producing 100 thousand tons in 68 thousand ha in 2020 (FAOSTAT, 2022).

Selenium is an element that is gained relevance for its benefits to human health. Also, common beans and quinoa are healthy foods of extraordinary nutritional quality; consequently, their consumption is increasing and contributes to food security worldwide. Considering that Se biofortification via soil is a suitable agricultural practice for enriching edible plants with Se bioavailable and ensuring human intake of a Se-rich diet. In addition to Se giving an additional benefit as an antioxidant increasing plants' tolerance to face water deficit, and common beans and quinoa are crops of social and economic importance for their corresponding representative regions. Our study aims to determine the effect of the Se application in soil on the Se-enrichment of common bean and quinoa grains and to evaluate the impact of the Se application

on the physiological and biochemical processes associated with tolerance to water deficit. The results of this research are reported in three papers:

Article 1 – Selenium application influenced selenium biofortification and physiological traits in water-deficit common bean plants

Journal: Crop & Pasture Science

Special issue: Mineral Biofortification and Metal/Metalloid Accumulation in Food Crops

DOI: 10.1071/CP20519

Article 2 - Soil selenium addition for producing Se-rich quinoa and alleviating water deficit on the Peruvian coast

Special Issue: Closing Yield Gaps on Hostile Soils

Manuscript with minor revisions submitted to Journal of Soil Science and Plant Nutrition

Article 3 - Soil selenium biofortification to enhance nutritional and physiological responses of quinoa crop grown under water deficit in an Oxisol

Ready to be submitted to Journal of Food Composition and Analysis

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SECOND PART – ARTICLES

ARTICLE 1: SELENIUM APPLICATION INFLUENCED SELENIUM BIOFORTIFICATION AND PHYSIOLOGICAL TRAITS IN WATER-DEFICIT COMMON BEAN PLANTS

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Abstract. Agronomic biofortification with selenium (Se) maybe employed to improve the nutritional value of food crops while increasing the plant's tolerance to water deficit conditions. Although not essential for plants, Se may increase plant tolerance to water stress by boosting plants' defence system activity. This study aimed to enrich common bean grains with Se and alleviate water deficit effects on common bean by applying Se to plants growing under greenhouse. Selenium was applied to soil at 0, 0.25, 0.5, 1.0, and 2.0 mg kg^{-1}, under irrigated and water-deficit conditions. Antioxidant enzyme activities (SOD, CAT, and APX), H_2O_2 content, gas exchange, and SPAD index were assessed in plant leaves, and Se and nutrient concentrations were determined in grains. Results showed that water deficit decreased photosynthetic rate, stomatal conductance, transpiration rate, and increased water use efficiency. Selenium did not influence SPAD index in leaves from pod formation to maturity stage but improved the plant defence system by decreasing H_2O_2 content. Increasing Se rates increased Se concentration in grains and leaves. At lower Se rates, mainly at 0.25 mg Se kg^{-1}, under water deficit, the activities of SOD, CAT, and APX reached their maximum values, and H_2O_2 content was minimum without reducing biomass production and nutrients accumulation. The highest Se rate (2 mg kg^{-1}) was detrimental, since it decreased biomass production and the plant defence system under water deficit. Conclusively, soil Se addition enriched common bean plants with Se and showed positive responses against water deficit when applied at lower rates.

Introduction

Water scarcity is an obvious problem that affects the food security and economy worldwide. Water deficit impacts morphological, physiological, and biochemical processes of crops at different degrees depending on the species and their phenological stages (Farooq *et al.* 2012; Sedlar *et al.* 2019; Dai *et al.* 2020; Farooqi *et al.* 2020; Huang *et al.* 2020). In fact, drought has become the main natural threat to crop production, with up to 80% of agricultural activity being affected. Sustainable agricultural practices may ensure sustainable food production, based on the efficient use of resources (Benfield 2017; FAO 2017, 2019; Farooqi *et al.* 2020).

Although food crops may have nutrients and vitamin that are ingested by humans, agronomic biofortification, which is a technological alternative to add nutritional value for crops through fertilisation, has been applied for improving the nutritional value of foods. This fact is particularly relevant for selenium (Se), which is not essential for plants but is for humans, especially in regions where Se contents in soils low, as in Brazilian tropical soils from the Cerrado biome (Carvalho *et al.* 2019). Biofortification with Se increased Se content successfully in a wide range of staple crops, as in wheat (Lara *et al.* 2019), rice (Boldrin *et al.* 2013; Lessa *et al.* 2020), and potatoes (de Oliveira *et al.* 2019) grown in tropical soils, which are highly weathered soils with high Se adsorption capacity (Mouta *et al.* 2008; Lessa *et al.* 2016; Araujo *et al.* 2020). For rice, field studies in Brazil have shown possibilities for producing rice grains with high Se levels to guarantee adequate Se intake by humans (Lessa *et al.* 2020). However, there are few studies reporting agronomic biofortification of legume crops, such as common bean grown in tropical soils. de Figueiredo *et al.* (2017) assessed under hydroponic condition the biofortification of common bean grains with Zn and Se, and how biofortified grains affected Fe bioavailability. The thesis of Namorato (2019) and de Araújo (2020) showed plant positive responses to Se-enriched fertilisers application in tropical soils, in terms of enriching the common bean grains with Se; however, they did not evaluate physiological analyses and the water deficit influence.

Although Se is not an essential element for plants (Winkel *et al.* 2015), it may promote the activation of their defence system. Selenium stimulates plant growth and mitigates production losses under environmental stresses such as water deficit (Aissa *et al.* 2018). Such benefit of Se supply for crops under water deficit can be attributed to the fact that Se increases the activity of antioxidant enzymes as well as of nonenzymatic antioxidants, reducing the presence of oxygen-reactive species (ROS) (Ahmad *et al.* 2016*b*; Ashraf *et al.* 2018). The protective role of Se against biotic and abiotic stresses involves several metabolic mechanisms,

with many of them being yet unclear. Recent investigations with rice (Andrade *et al.* 2018) and sweet potato (Huang *et al.* 2020) under water deficit conditions and with potato cultivated in tropical soil (de Oliveira *et al.* 2019) have reported synergistic effects of Se and antioxidant enzyme activities to control ROS by protecting chloroplast membranes and regulating the photosynthetic system. Besides the fact that the production of Se-rich grains is relevant for nutritional purposes, the possible positive effects of Se application in plant development under stress conditions (e.g. water deficit) may be another important reason for convincing producers to employ agronomic biofortification with Se. In this context, studies evaluating the effect of adding Se on biofortification with Se and tolerance to water deficit are required especially on legumes (e.g. common bean) grown in tropical soils.

Common bean (*Phaseolus vulgaris* L.) is the most important food legume that is cultivated worldwide. Brazil is one of the biggest producers, harvesting 2.91 million tons from 2.61 million ha in 2019 (FAOSTAT 2012). The common bean grain is consumed directly by humans and provides high levels of protein, carbohydrates, vitamins, and minerals. Given the great nutritional contribution of the common bean and its consumption, agricultural strategies are focussed on increasing its productivity and nutritional value. Moreover, sustainable common bean production is achieved by an adequate supply of water and nutrients (FAO 2018).

Taking into account the importance of common bean in population diets and the protective action of Se in plants (Gupta and Gupta 2017; Natasha *et al.* 2018), the hypothesis raised for the present study is that Se may increase the nutritional quality of common bean grains and also improve the plant's tolerance to water deficit. Research to assess the biofortification potential of common bean with Se and its key role against the effects of low water availability in soils are relevant but not well known. The most similar study found in the literature was with soybean, where the biofortification with Se and Zn increased soybean physiological functions (Dai *et al.* 2020). Therefore, this research employed agronomic biofortification of common bean with Se, aiming to (i) enrich the common bean grains with Se, and (ii) activate the defence system of the plant to avoid or alleviate the negative impact of water deficit. Considering that the dose–response relationship of Se may trigger beneficial effects at low rates and toxic or detrimental effects at high rates, we compared different Se rates to select the best Se rate that, besides enriching common bean grains with Se, improves the efficacy of the plant defence against water deficit.

Material and methods

Soil description and experimental design

For this study, a pot experiment was conducted in a greenhouse located at the Soil Science Department, Federal University of Lavras (UFLA), Lavras, State of Minas Gerais, Brazil, from September to December 2019. The amount of soil needed for the whole experiment was collected from the 0–20 cm depth in an Oxisol profile from a secondary forestry located at the UFLA campus (12° 04' 54.5" S, 76° 57' 08.3" W, 968 m above sea level) (Curi *et al.* 2017), air-dried, and sieved through a 4-mm mesh. Next, a composite soil sample was analysed following the methodologies suggested by the Brazilian Co. of Agricultural Research (EMBRAPA) (Teixeira *et al.* 2017), and the main soil characteristics were as follows: pH in water = 4.60; soil organic matter = 32.70 g kg^{-1}; available phosphorus (P) by Mehlich-1 = 1.18 mg kg^{-1}; available potassium (K) = 61.90 mg kg^{-1}; exchangeable calcium (Ca) = 0.45 cmol$_c$ kg^{-1}; exchangeable magnesium (Mg) = 0.28 cmol$_c$ kg^{-1}; exchangeable aluminum (Al) = 1.18 cmol$_c$ kg^{-1}; total acidity (H+Al) = 11.62 cmol$_c$ kg^{-1}; cation exchange capacity (CEC) at pH 7 = 12.51 cmol$_c$ kg^{-1}; clay = 610 g kg^{-1}; silt = 110 g kg^{-1}; and sand = 280 g kg^{-1}. Total soil Se content was 0.27 mg kg^{-1}, which was determined after acid digestion according to the USEPA (2007) 3051A method followed by Se determination by atomic absorption spectrometry with graphite furnace atomisation (GFAAS) (AAnalyst™ 800 AAS, Perkin Elmer).

Pots were filled with 5 L of soil (bulk density = 1.03 g cm^{-3}). Liming was carried out based on soil analysis to reach a base saturation of 65%, using limestone with a CaCO$_3$/MgCO$_3$ ratio of 4:1, which was incorporated and incubated for 40 days, with soil moisture content being kept close to 50% (w/w) of the water holding capacity. Fertilisation consisted of adding the following nutrient amounts (in mg kg^{-1}): 300 of N, 200 of P, 150 of K, 50 of S, 0.5 of B, 1.5 of Cu, 0.1 of Mo, and 5.0 of Zn (Malavolta 1981). Fertilisation with N and K was split to three and two applications, respectively. The first N and K application along with all other nutrients was performed before sowing; the second N and K application happened at 20 days after sowing (DAS) when the third trifoliate leaf expanded (V4 stage); and the third application of N was carried at 45 DAS, during the pod formation stage (R7 stage). Details regarding the terminologies used for phenological stages of the common bean crop were described by Meier (2001) in the Biologische Bundesanstalt Bundessortenamt und Chemische Industrie (BBCH) scale, which Oliveira *et al.* (2018) divided into two biological cycles: vegetative phase (from V0 to V4) and reproductive phase (from R5 to R9).

The different Se rates - 0.00, 0.25, 0.50, 1.00, and 2.00 mg kg^{-1} - were applied in the soils as sodium selenate (Na$_2$SeO$_4$, Sigma-Aldrich, St Louis, MO, USA) before sowing.

Common bean plants received these Se additions at two soil water conditions: under continuous irrigation, hereafter called irrigated (I), for simplicity, and under water deficit (WD). Therefore, the experiment was set up as a completely randomised design in a 5 x 2 factorial scheme with four replicates.

Five *Phaseolus vulgaris* L. CV BRS Estilo seeds, a cultivar of high productive potential (Cargnin and Albrecht 2010), were sown in each pot. Then, thinning was carried out to two plants per pot at two-leaf stage.

Establishment of water deficit conditions

Initially, all pots were at a moisture content equivalent to the water holding capacity, which was determined before starting the experiment by weighing the pots after the soil was saturated and the water excess drained. The water treatments were imposed 35 days after plant emergence. Pots under water deficit were not irrigated for 6 days from the stage when 50% of plants were flowering (R6 stage – 35 days after emergence) to the stage within the pod formation (R7 stage – 41 days after emergence). This period when plants were exposed to water deficit is considered the most sensitive phenological stage to water stress for the common bean crop (Mathobo *et al.* 2017; Oliveira *et al.* 2018; Cavalcante *et al.* 2020). Water deficit was measured by the fraction of transpirable soil water (FTSW) following previous studies (Sinclair and Ludlow 1986; Ray and Sinclair 1997; Jacobsen *et al.* 2009), as it is shown in the supplementary material (Supplementary material Fig. S1, available at the journal's website). Soil evaporation was minimised by enclosing the pot inside a sealed plastic bag. Plants under water deficit absorbed the available water in the pot until their transpiration rate was equivalent to 10% of the transpiration rate of the irrigated treatments. This transpiration rate was achieved on the sixth day of water deficit, where the evaluation of gas exchange and SPAD index, as well as leaves collection for enzymatic and H_2O_2 analyses, were carried out. Next, plants were rewatered to recover them from the stress caused by the water deficit, which allowed them to keep their development until harvest.

Gas-exchange measurements

Gas exchange was measured on the last day of the water deficit (i.e. 41 days after emergence, during the pod formation stage) between 8:00 and 11:00 hours. The density of the photosynthetically active photon flux was fixed in the device chamber at 1000 μmol m^{-2} s^{-1}, the

vapour pressure deficit was 2.66 kPa, the pump flow was 500 μmol s^{-1}, and the block temperature was 25°C. A portable infrared gas analyser (IRGA, model LICOR 6400, Li-COR Biosciences, Lincoln, NE, USA) was used to measure the photosynthetic rate (A, μmol CO_2 m^{-2} s^{-1}), the stomatal conductance (g_s, mol H_2O m^{-2} s^{-1}), and the transpiration rate (E, mmol H_2O m^{-2} s^{-1}). Photosynthetic and transpiration rates were used to estimate water use efficiency (WUE, (A/E, μmol CO_2 mmol^{-1} H_2O)). These assessments were recorded in the fully expanded leaf at pod formation stage.

SPAD index measurements

The SPAD chlorophyll index, hereafter termed SPAD index, was assessed on the same day and in the same leaf used for gas exchange measurements, using a portable chlorophyll meter (SPAD-502, Konica-Minolta, Japan). The value considered for the SPAD index was the average of three regions of each leaf (basal, medium, and apical region), the regions in which three measures were also obtained to calculate the average value per region. Additional assessments of the SPAD index were also made on representative leaves at stages V3 (first trifoliate leaf) and V4 (third trifoliate leaf).

Antioxidant enzyme activities and hydrogen peroxide (H_2O_2) quantifications

Antioxidative enzyme activities and hydrogen peroxide concentration were quantified in the same leaf where gas exchange and the SPAD index were measured (41 days after emergence). Fresh leaves were collected on the sixth day of water deficit and immediately placed into liquid nitrogen to preserve their biochemical condition; then, they were stored at -80°C until analysis. Fresh frozen leaves were ground in liquid nitrogen on a pre-chilled mortar, and polyvinylpolypyrrolidone (PVPP) was added to complete maceration.

Briefly, as described by Biemelt *et al*. (1998), 0.2 g of macerated samples were homogenised in 1.5 mL of extraction buffer solution (100 mM potassium phosphate at pH 7.8, 0.1 mM EDTA, and 10 mM ascorbic acid), centrifuged at 13 000g for 10 min at 4°C, and the supernatant was collected for further enzyme activity assessments. Enzyme extract aliquots were used for measuring the activities of the different enzymes using different methods, as follows: superoxide dismutase (SOD) (Giannopolitis and Ries 1977), catalase (CAT) (Havir and McHale 1987), and ascorbate peroxidase (APX) (Nakano and Asada 1981). Additionally, 0.3 g of macerated samples were homogenised in 1.5 mL of 0.1% trichloroacetic acid (TCA),

centrifuged at 12000g for 15 minutes at 4°C, and the supernatant was collected for quantifying the concentration of hydrogen peroxide (Velikova *et al.* 2000) by Epoch™ Microplate Spectrophotometer (Biotek, US).

Biometric measurements and chemical analysis

Common bean grains were harvested at the end of the crop cycle, with a moisture content of ~11%. Plant shoots were collected and separated into leaves, stems, and pods for further analysis. Leaves, stems, and grains were dried in a forced-air oven at 70°C for 48 h to obtain their dry weight.

The dry mass of leaves and grains was digested in nitric acid according to the USEPA 3051A method to determine their elemental concentrations (USEPA 2007). Nitrogen concentration was determined by digestion with sulfuric acid and titration by the Kjeldahl method (Malavolta *et al.* 1997). Phosphorus, K, Ca, Mg, and S concentrations were measured by ICP-OES, whereas Se was determined by atomic absorption spectrometry with graphite furnace atomisation (GFAAS) (AAnalyst™ 800 AAS, Perkin Elmer). The QA/QC protocol for Se analyses was assured with the addition of two standard/certified reference materials – White Clover-BCR402 (Institute for Reference Materials and Measurements – IRMM, Geel, Belgium) and Peach Leaves-SRM 1547 (National Institute of Standards and Technology – NIST, Gaithersburg, MD, USA) – in each batch of digestion, with Se recovery values ~95%.

Selenium-recovery by grains was calculated by the difference in Se accumulation between the treatments with Se addition and the control, divided by the amount of Se applied in the respective treatments, as follow (adapted from Lessa *et al.* 2019):

$$\text{Se-recovery} = ((\text{Se}_{treat} - \text{Se}_{control}) / \text{Se}_{pot}) \times 100$$

where, Se-recovery (%) = Se use efficiency by common bean grains for each specific Se rate applied in the soil, Se_{treat} (mg pot^{-1}) = Se accumulated by grains in each treatment (Se rate), $\text{Se}_{control}$ (mg pot^{-1}) = Se accumulated by grains in the control treatment, Se_{pot} (mg pot^{-1}) = amount of Se applied per pot.

Statistical analysis and data processing

Data obtained were analysed using programming language R (R Core Team 2019). Analysis of variance was performed, and treatment means were compared by Tukey's test at α = 0.05. Also, the combined effect of treatments on variables was evaluated through principal

component analysis (PCA). Graphics were elaborated, and regression models were fitted with the SigmaPlot for Windows software, ver. 11.0 (Systat Software Chicago, IL, USA).

Results

Gas exchange, antioxidant enzyme activities, and hydrogen peroxide concentration

Soil water conditions significantly ($P \leq 0.05$) affected gas exchange, irrespectively of the Se addition. Water deficit condition reduced photosynthetic rate (A), stomatal conductance (g_s), and transpiration rate (E) (Fig. 1a-c), and increased the water use efficiency (WUE) (Fig. 1d). On average, water deficit condition decreased A, g_s, and E by 68, 95, and 91%, respectively, and promoted an increase of 318% in WUE.

Antioxidant enzyme activities and hydrogen peroxide (H_2O_2) concentration were significantly ($P \leq 0.05$) influenced by Se rates (Fig. 2a-d). More specifically, the activity of superoxide dismutase (SOD) did not express clear differences in the studied treatments (Fig. 2a), whereas catalase (CAT) (Fig. 2b) and ascorbate peroxidase (APX) (Fig. 2c) activities were more sensitive to the applied Se rates. CAT activity was higher when Se was added at 0.25 and 2 mg kg^{-1} for irrigated plots and at 0.25, 0.5, and 2 mg kg^{-1} for water deficit plots (Fig. 2b). APX activity for irrigated plots increased with the application of 0.25 and 0.5 mg kg^{-1} of Se, subsequently decreasing to the same level of the control at higher Se rates (1 and 2 mg kg^{-1}). Under water deficit condition, APX activity was higher when Se was applied at 0.25 and 1 mg kg^{-1} (Fig. 2c). Hydrogen peroxide concentrations did not change with the application of Se, except for the highest Se rate (2 mg kg^{-1}) under water deficit, where H_2O_2 content increased significantly (Fig. 2d).
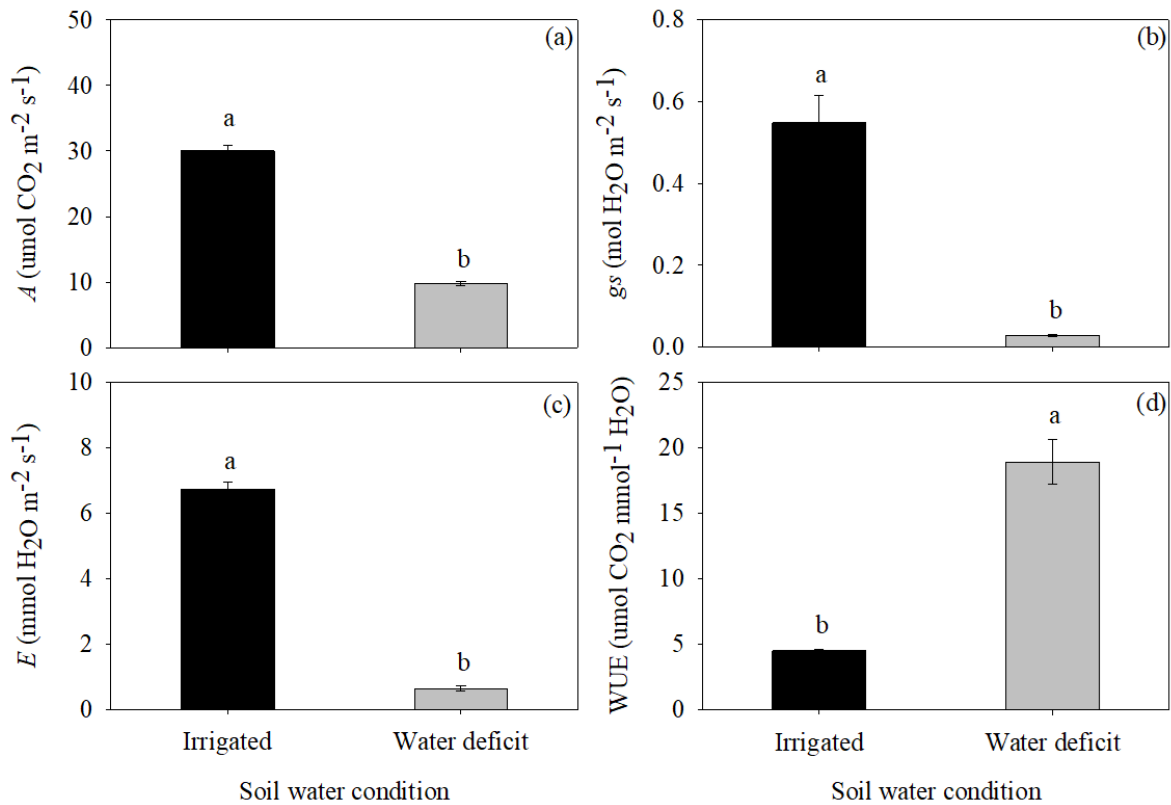
Fig. 1. Photosynthetic rate (*a*), stomatal conductance (*b*), transpiration rate (*c*), and water use efficiency (*d*) in leaves of common bean after water deficit. Lowercase letters compare the means of soil water conditions, irrespectively of Se rates, according to Tukey's test (at 5% probability). The interaction between Se rates and soil water condition was not significant. Vertical bars indicate s.e. of average values (*n = 20*).
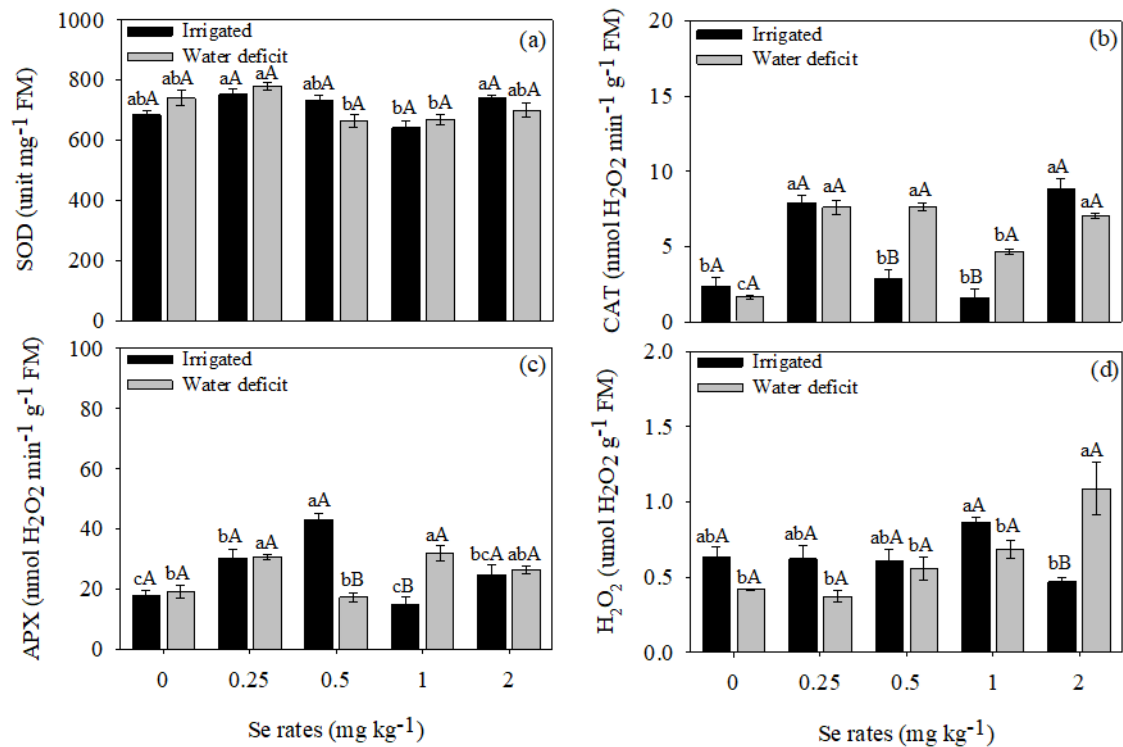
Fig. 2. Enzymatic activity of superoxide dismutase (SOD) (*a*), catalase (CAT) (*b*), and ascorbate peroxidase (APX) (*c*), and concentration of hydrogen peroxide (*d*) in the fresh mass (FM) of common bean leaves. Uppercase letters compare the means of soil water conditions at each Se rate, and lowercase letters compare the means of Se rates at each soil water condition, according to Tukey's test (at 5% probability). Vertical bars indicate s.e. of average values (*n = 4*).

SPAD chlorophyll index

SPAD index measured on leaves on the last day of exposing plants to water deficit (R7 stage) varied ($P \leq 0.05$) depending on soil water conditions, with values 20% higher for plots under water deficit compared with irrigated ones (Fig. 3a). SPAD index measured during the R7 stage was not influenced by the Se application. However, changes in this index were observed when it was measured in earlier stages (V3 and V4). Leaves of the R7 stage registered the lowest values of the SPAD index.

The interaction between the studied factors (water condition and Se rates) influenced (*P* ≤ 0.05) SPAD chlorophyll index in leaves of V3 and V4 stages (Fig. 3b, c). Selenium rates did not modify the SPAD index measured on leaves from the V3 stage in irrigated plots but affected it in the water deficit condition. The SPAD index under water deficit was constant up to 1 mg kg^{-1} Se and dropped at the highest rate by 34% compared with the control treatment and 40% concerning the same treatment in the irrigated condition (Fig. 3b). Similar trends for SPAD index recorded in leaves from the V4 stage were observed compared with what was mentioned

for the measurements during V3 stage, yet the application of Se at 2 mg kg^{-1} also decreased the SPAD index in irrigates condition compared with the lower rates (Fig. 3c).
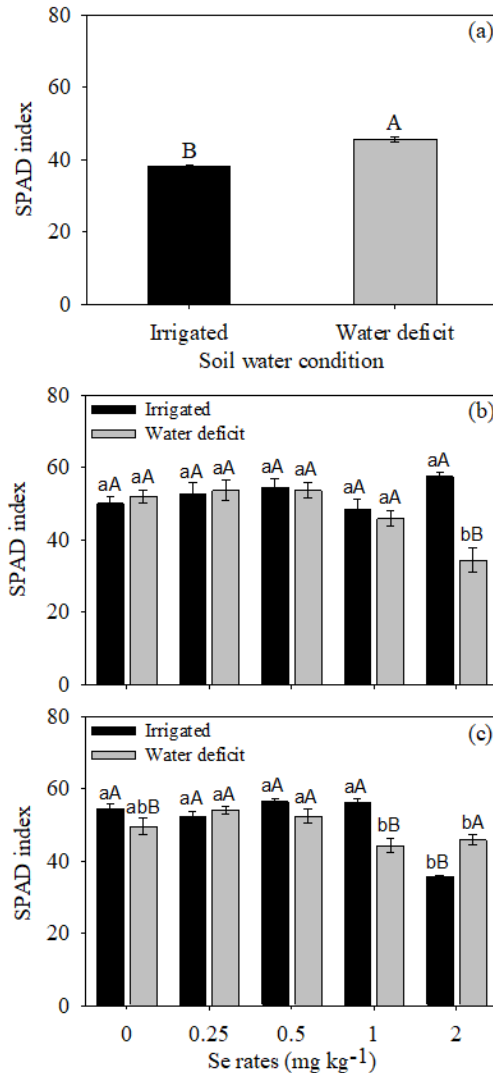
Fig. 3. The SPAD index on leaves from R7 stage (*a*) and additional assessments on leaves from V3 (*b*) and V4 (*c*) stages of common bean after water deficit. Uppercase letters compare the means of soil water conditions at each Se rate, and lowercase letters compare the means of Se rates at each soil water condition, according to Tukey's test (at 5% probability). Vertical bars indicate s.e. of average values: (*a*) *n = 20*; (*b*) *n = 4*, and (*c*) *n = 4*.

Dry mass

Shoot and grain dry mass productions (Fig. 4a, b) were influenced ($P \leq 0.05$) by the interaction between the studied factors (water condition and Se rates). In the grain (Fig. 4a), Se supply on irrigated treatments did not modify the dry mass production. Under water deficit, grain dry mass production at the highest Se rate (2 mg kg^{-1}) reduced by 40% compared with its control and by 47% when compared with the same Se rate in the irrigated condition.

The highest Se rate applied decreased the shoot dry matter in irrigated and water deficit treatments by 20 and 10%, respectively (Fig. 4b). Under the irrigated condition, dry mass tended to be higher. Dry mass production by leaves and stem was also recorded, and data showed similar trends to what was reported for the shoot (data not shown).
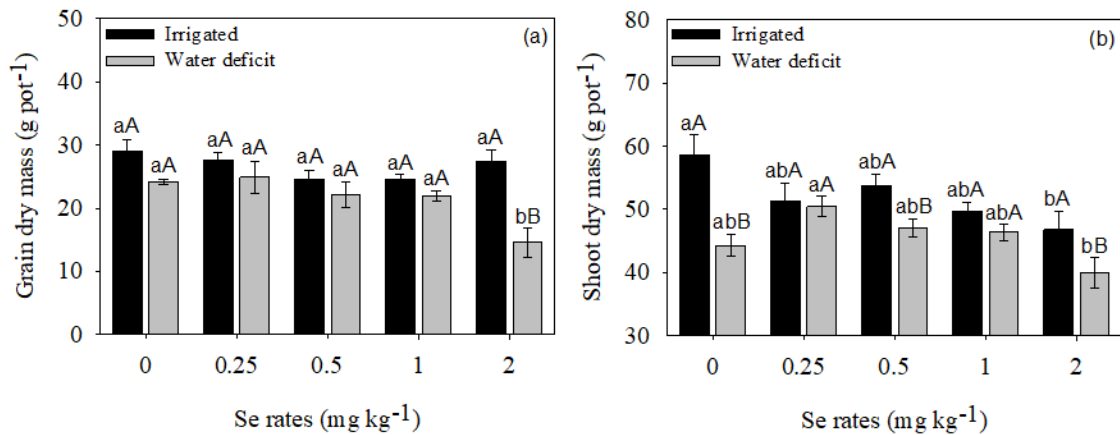
Fig. 4. Production of grain dry mass (*a*) and shoot dry mass (*b*) of common bean. Uppercase letters compare the means of soil water conditions at each Se rate, and lowercase letters compare the means of Se rates at each soil water condition, according to Tukey's test (at 5% probability). Vertical bars indicate s.e. of average values (*n = 4*).

Selenium concentration and accumulation, and Se recovery percentage

The interaction between Se rates and soil water conditions affected also ($P \leq 0.05$) the concentration of Se in grains and leaves of common bean (Fig. 5a, b). Selenium contents in grains (Fig. 5a) and leaves (Fig. 5b) at irrigated and water deficit treatments increased linearly upon increasing the Se rates applied ($R^2 \geq 0.91$). Comparing soil water conditions, Se contents in grains at the water deficit plots tended to be higher than that at irrigated treatments. Conversely, Se contents in leaves were higher in irrigated plots compared with the ones under water deficit. In both soil water conditions, Se contents were significantly different at the highest rate (2 mg kg^{-1}), where Se contents in grains and leaves increased and decreased, respectively, by 32% and 28% under the water deficit condition.

Selenium accumulation by grains (in mg per pot) (Fig. 6a) as a function of Se rates added was also explained by a linear equation, as reported for Se content. This accumulation for water deficit treatments was lower than that detected for irrigated treatments, dropping by 45% when Se was added at 2 mg kg^{-1}. Accumulations of N, P, K, Mg, and S by grains showed quadratic equations for deficit and irrigated treatments, and their water deficit treatments accumulated lower amounts of the nutrient itself as found by Se (Fig. S2).

Selenium recovery percentages (Fig. 6b) as a function of Se rates added followed quadratic equations for both soil water conditions. Se-recovery by grains at irrigated plots was higher than for the water deficit condition, which was also observed for Se accumulation. The water deficit imposed during 6 days affected Se-recovery of treatments receiving Se rates greater than 0.5 mg kg^{-1}. Under the irrigated condition, the addition of Se at 1 and 2 mg kg^{-1} reduced Se-recovery percentage by 17 and 45% compared with these same Se rates applied under the water deficit condition.

Based on the equations presented on Fig. 6a, b, at Se rates greater than 0.3 mg kg^{-1}, Se accumulation efficiency of irrigated treatments is higher than that of water deficit treatments. Selenium rates above 1.25 mg kg^{-1} begin to decrease Se recovery efficiency by grains under water deficit conditions.
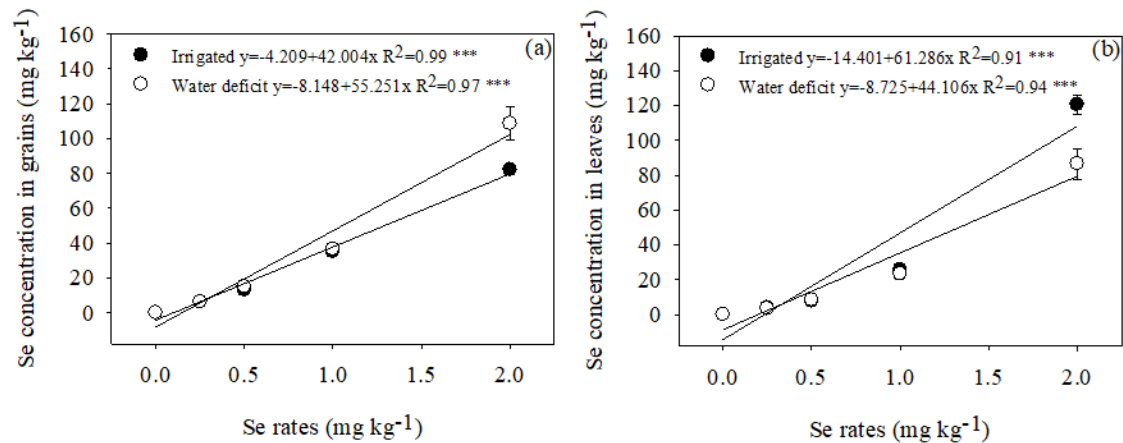
Fig. 5. Selenium concentration in grains (*a*) and leaves (*b*) of common bean concerning Se rates and soil water conditions. Vertical bars indicate s.e. of average values (*n = 4*). Statistically significant differences are indicated: ***, $P \leq 0.001$.
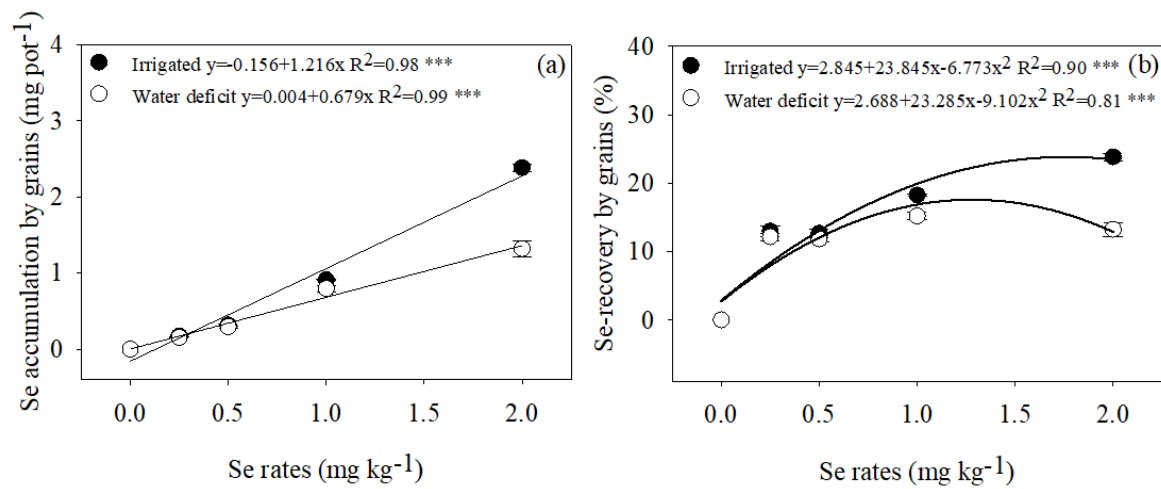
Fig. 6. Selenium accumulation by grains (*a*) and selenium recovery by grains (*b*) of common bean concerning Se rates and soil water conditions. Vertical bars indicate s.e. of average values (*n = 4*). Statistically significant differences are indicated: ***, $P \leq 0.001$.

Principal component analysis (PCA) of individuals

The analysis by principal components of individuals (Fig. 7), based on the Euclidean distance, has evidenced four distinct groups among the samples. Overall, it is possible to detect that the four excluding groups enclosed in the ellipses are composed of samples from the following treatments: irrigated treatments from 0 to 1 mg kg^{-1} Se, water deficit treatments from 0 to 1 mg kg^{-1} Se, irrigated treatment with 2 mg kg^{-1} Se, and water deficit treatment with 2 mg kg^{-1} Se.
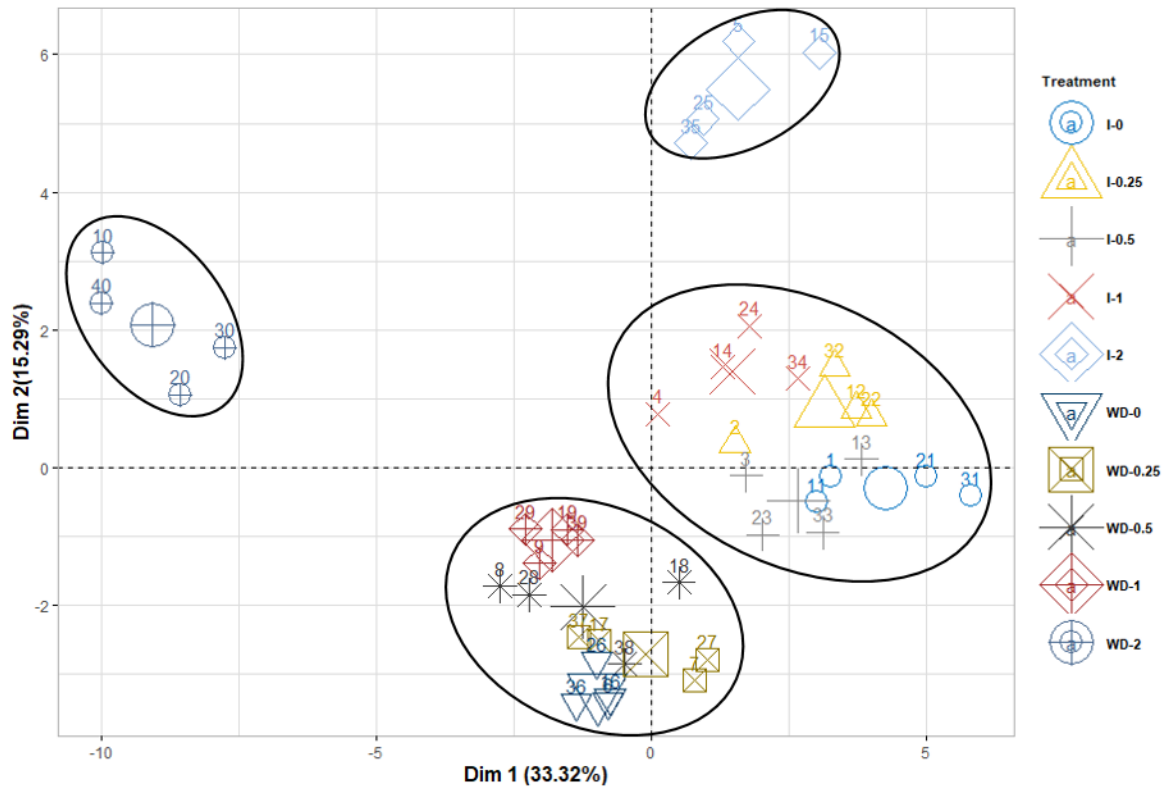
Fig. 7. PCA graph of individuals. Symbols and numbers of the same colour represent the treatments of soil water conditions (irrigated (I) and water deficit (WD)) in each Se rates (0.00, 0.25, 0.50, 1.00, and 2.00 mg kg^{-1}).

Discussions

Gas exchange, antioxidant enzyme activities, and hydrogen peroxide concentration

Our data confirmed that common bean faced a severe water deficiency since stomatal conductance was lower than 0.05 mol H_2O under the water deficit condition (Fig. 1b), according to the values reported by Flexas *et al.* (2012) and Mathobo *et al.* (2017). Based on results of *A*, *g$_s$*, and *E* (Fig. 1a-c), the application of Se could not mitigated water stress in the present study. The water deficit level was probably too high and because of that gas exchange measurements were only affected in response to soil water conditions. For gas-exchange traits, the hormesis effect of Se might be nullified by the severe water deficiency experienced by common bean plants, a condition aggravated by the susceptibility of the cultivar BRS to water deficit. Since Se alleviated the oxidative stress caused by water deficit, we might suppose that the response time did not allow the expression and/or the regulation of the antioxidant defence system at a level that would protect chloroplast membranes, positively interfering in gas exchange. Certainly, a metabolic response at the photosynthetic process level depends on intensity and

time of exposure to stress. Huang *et al.* (2020) showed that Se rates enhanced gas exchange in sweet potato leaves for their different periods with water deficits.

Water use efficiency (Fig. 1d) increased under the water deficit condition as a consequence of the lower photosynthetic rate (Fig. 1a) and stomatal conductance (Fig. 1b), and the decrease in water loss (Fig. 1c), as reported elsewhere (Flexas *et al.* 2012; Ahmad *et al.* 2016*a*; Yan *et al.* 2016; Mathobo *et al.* 2017; Andrade *et al.* 2018; Sedlar *et al.* 2019; Huang *et al.* 2020).

Fig. 2 shows the dual effect of Se on common bean plants, i.e. beneficial effects until a certain rate of Se addition and adverse ones when Se was added in higher rates. The antioxidant defence system of common bean plants evidenced changes due to Se protective action, while these Se rates applied did not affect gas exchange.

Increases in SOD activity demonstrated the positive effect of Se application in rice (Andrade *et al.* 2018), sweet potato (Huang *et al.* 2020) and potato (de Oliveira *et al.* 2019), irrespectively of the plant species. Common bean in this research showed slight differences in SOD activity (Fig. 2a), as it was found by Lessa *et al.* (2020) in rice. However, some studies reported no Se effect upon SOD activity (Lara *et al.* 2019).

In contrast to what was found for SOD, the activities of CAT and APX varied with Se addition (Fig. 2b, c), being higher than at 0 to 0.5 mg Se kg^{-1}. Similar findings were also reported by de Oliveira *et al.* (2019) and Huang *et al.* (2020) for CAT activity, and by Lara *et al.* (2019) for APX activity. Water deficit might trigger the formation of H_2O_2 stimulating the action of enzymes that control it instead of forming the superoxide ion. CAT and APX act directly on H_2O_2 as scavengers and their activities might also reduce the formation of superoxide anion radicals (Farooq *et al.* 2012; Flexas *et al.* 2012). Considering that the H_2O_2 concentrations were similar at Se rates below 2 mg kg^{-1} (Fig. 2d), CAT and APX activities changed mainly in response to Se rates applied, as reported by Lara *et al.* (2019) for wheat.

Low Se rates (0.25 and 0.5 mg kg^{-1}) improved the plant's defence system by increasing antioxidant enzyme activities. Under water deficit conditions, this defence system acted in the oxidative stress by reducing the production of ROS (Farooq *et al.* 2012; Flexas *et al.* 2012; Aissa *et al.* 2018; Hawrylak-Nowak *et al.* 2018; de Oliveira *et al.* 2019; Farooqi *et al.* 2020). The higher Se rate (2 mg kg^{-1}) was detrimental since it magnified the oxidative stress level caused by the water deficit, as reported by de Oliveira *et al.* (2019). In sum, Se additions ≤ 1 mg kg^{-1} were beneficial to stimulate CAT and APX, but higher rates adversely affected the efficacy of the antioxidant defence system of common bean plants, with peroxide concentrations increasing.

SPAD chlorophyll index

SPAD index was affected by the age of leaves (Fig. 3a-c), as reported in other studies (Doğru and Çakirlar 2020; Obiero *et al.* 2020). In general, after exposure to water deficit, younger leaves from V3 (Fig. 3b) and V4 stages (Fig. 3c) (vegetative stages) showed lower SPAD index values than that verified for leaves of the R7 stage (Fig. 3a) (reproductive stage). This indicates that the SPAD index has changed depending on the crop cycle (Mathobo *et al.* 2017). According to Sedlar *et al.* (2019), the variation in chlorophyll content in leaves from different ages could be related to the abundance of proteins associated with the photosynthetic process as a mechanism to hold homeostasis and to tolerate water deficit.

Antioxidant enzyme activities have confirmed that Se may mitigate the oxidative stress at the antioxidant defence system level, and the quantity of ROS, in this case, was not enough to affect chlorophyll content. However, a higher value of SPAD index under water deficit responded better to a protective mechanism by age leaf or the type of crop (Doğru and Çakirlar 2020).

Chlorophyll is an important photosynthetic pigment that absorbs solar radiation to drive photosynthesis, and its content in the leaf is considered an indicator of photosynthetic capacity. Water deficits restrict carbon dioxide fixation leading the plant to activate selected mechanisms to maintain the photosynthetic capacity to face this condition (Farooq *et al.* 2012; Aissa *et al.* 2018; Sun *et al.* 2020).

After applying the higher Se rates, leaves from V3 (Fig. 3b) and V4 (Fig. 3c) stages showed lower values for SPAD index, which means that plants decreased their photosynthetic rate. This result might be related to the greater oxidative stress caused by adverse effects of the higher Se rates and the severe water deficit. Both promoted reactive oxygen species (ROS) production in chloroplasts, the primary places to them. ROS accumulation affects this organelle by decomposing chlorophyll and lessening chlorophyll content, and consequently, chloroplast membranes and thylakoid structures are destructed. This oxidative stress altered the common bean's metabolic processes and, as a consequence, the photosynthetic rate dropped (Flexas *et al.* 2012; Mathobo *et al.* 2017; Sedlar *et al.* 2019). Selenium toxicity induced lipid peroxidation due to the increase in ROS production, and consequently, harms cell membrane and proteins (Aggarwal *et al.* 2011; Gupta and Gupta 2017; Natasha *et al.* 2018; White 2018; Cabral Gouveia *et al.* 2020). Elkelish *et al.* (2019) found also that higher Se concentration diminished the growth and synthesis of chlorophyll pigments.

Low Se rates at leaves of V3 (Fig. 3b) and V4 (Fig. 3c) probably due to the protective function of Se, which avoided oxidative stress by scavenging ROS, while also maintaining chloroplast structures and their photosynthetic activity (Huang *et al.* 2020).

Dry mass

Photosynthesis uses light energy to synthesise organic compounds in leaves from inorganic matter to form plant biomass. Under water deficit, the decline of dry mass accumulation in the grain and shoot of the common bean (Fig. 4 a, b) could be attributed to lower photosynthetic efficiency, as results in the gas exchange have evidenced. Likewise, the marked difference between dry matter in the grains and shoot can be explained by the type of organ and plant age, as highlighted for different crops elsewhere (de Figueiredo *et al.* 2017; Mathobo *et al.* 2017; Andrade *et al.* 2018; Dai *et al.* 2020). Under water deficit, plants diminish their foliar area destined for the photosynthetic process. As a consequence, biomass production per unit area, growth, and crop yield are reduced, although this depends on the species (Ahmad *et al.* 2016*a*). At the same time, the crop growth cycle is negatively affected because the phenological stages are shortened (Farooq *et al.* 2012; Farooqi *et al.* 2020). In our study, common bean had lower water consumption, yet the production of dry mass per unit of water consumed was greater. Its WUE increased because stomatal conductance adjusted CO_2 fixation and regulated metabolic pathways, hence ensuring higher productivity even under water deficit conditions (Farooq *et al.* 2012; Xu *et al.* 2015; Mathobo *et al.* 2017).

Selenium strengthens the resilience to abiotic stress through its beneficial actions on the plant defence system. Moreover, Se reinforces the continuity of metabolic processes by maintaining the cell integrity, thereby ensuring the growth of the crop and the harvest because photosynthesis and the formation of biomass are not interrupted (Gupta and Gupta 2017; Natasha *et al.* 2018; White 2018; Dai *et al.* 2020).

Under continuous irrigation conditions, the biomass production by common bean grains (Fig. 4a) suggested that the adequate water supply did not alter its nutrient transport, and when there was no stress condition, Se did not activate any protective system in the plant. The same behaviour was found at lower water availability as well, but the oxidative stress generated at the highest Se rate merged with water deficit, so that dry matter production lessened drastically since the crop is not a Se hyperaccumulator plant, as referred by Natasha *et al.*(2018). In contrast, according to Calabrese and Baldwin (2003), the dose-response relationship can be seen by the loss in biomass production.

Shoot biomass (Fig. 4b) with adequate water supply was reduced by Se addition at 2 mg kg^{-1}, whereas under water deficit, Se rates mitigated the water deficit and improved dry matter production, except for 2 mg kg^{-1}, a Se rate that led to harmful effects, as was found in rice by Cabral Gouveia *et al.* (2020). Obviously, this response depends on the species and the morphological differences among their organs, as well as on the role that different Se concentrations might have in specific plant species (de Figueiredo *et al.* 2017; Cabral Gouveia *et al.* 2020).

Selenium concentration and accumulation, and Se recovery percentage

Selenium uptake and accumulation by plants is causally related to soil Se content, Se speciation, and the metabolic process of the plant organ. In our study, Se contents in common bean grains and leaves increased after Se application, agreeing with other studies (Boldrin *et al.* 2013; de Figueiredo *et al.* 2017; Andrade *et al.* 2018; Lessa *et al.* 2019, 2020; Dai *et al.* 2020). However, some studies have not found a direct correlation between water-soluble Se and plant Se contents (Natasha *et al.* 2018).

The greater Se concentration from grains harvested under water deficit treatments (Fig. 5a), when compared with irrigated treatments may be justified by the concentration effect, since results indicated a reduction of biomass production under the water deficit condition, but not due to the presence of Se (Bocchini *et al.* 2018). Leaves showed smaller Se content under water deficit (Fig. 5b) because the defence system of the plant was promoted in such conditions, improving the efficiency of photosynthates translocation from leaves to grains to ensure the nutrient storage in the grain (Gupta and Gupta 2017; Natasha *et al.* 2018; White 2018; Huang *et al.* 2020), a fact observed for most nutrients, as shown in Fig. S2. Selenium transfer from soil-root-shoot to grains is controlled by Se speciation because chemical forms of Se are related to the metabolic process of the plant organs, with Se being accumulated mainly in shoot (Gupta and Gupta 2017; Natasha *et al.* 2018; White 2018; Dai *et al.* 2020).

The trend of Se accumulation (Fig. 6a) as a function of Se rates applied in both soil water conditions was similar, which was also observed by Andrade *et al.*(2018) and Dai *et al.* (2020). Selenium concentration was higher in the grains under water deficit compared with the irrigated treatments, with the behaviour of Se accumulation being the opposite due to the reduction of dry matter production in absence of water (Gupta and Gupta 2017; Natasha *et al.*2018).

Se-recovery results (Fig. 6b) were affected by Se availability in soils and by plant ability to absorb and transfer Se to grains. The trends of the Se-recovery curves are usually affected by high Se rates (Lessa *et al.* 2019). This could explain the quadratic responses observed for Se-recovery in the present study, since Se addition at higher rate may induce the oxidative stress that minimises Se uptake. This is also explained by the fact that, unlike some Se-hyperaccumulators, common bean plants have no tolerance mechanisms against Se excess, to alleviate damage from Se toxicity (Natasha *et al.* 2018). Se-recovery percentages oscillated between 12 and 24%, which exceed the 5% published by Natasha *et al.* (2018).

Principal component analysis (PCA) of individuals

The distribution of the groups showed in this study has reinforced the evidence of the hormesis effect (Fig. 7). Irrespectively of soil water condition, treatments that received lower Se rates (from 0 to 1 mg kg^{-1}) were grouped differently to what was verified for treatments where Se was applied at higher rates (2 mg kg^{-1}). Therefore, these lower and higher Se additions caused, respectively, beneficial and toxicity effects by harming common bean productivity, which was also found by Calabrese and Baldwin (2003), Natasha *et al.* (2018), de Oliveira *et al.* (2019), Cabral Gouveia *et al.* (2020), and Liang *et al.* (2020). Furthermore, most of the treatments within the same ellipse shown in PCA were statistically similar, as can be seen by the multiple comparison analysis of each variable.

Conclusions

Agronomic biofortification with increasing soil Se rates was effective to produce Se-rich common bean grains. Besides producing Se-rich common bean grains, when applied at lower rates (0.25 and 0.5 mg kg^{-1}), Se protected the plants against water deficit by increasing antioxidant enzyme activities, decreasing hydrogen peroxide. In contrast, Se addition at higher rates (1 and 2 mg kg^{-1}) had adverse effects, decreasing the common bean biomass production. Considering that the present study was carried out in greenhouse condition, with common bean being grown for one cropping season, further researches in field conditions are recommended to better define the Se rates to be applied and Se action mechanisms against water deficit.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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ARTICLE 2: SOIL SELENIUM ADDITION FOR PRODUCING SE-RICH QUINOA AND ALLEVIATING WATER DEFICIT ON THE PERUVIAN COAST

This article was prepared in line with the guidelines of the Journal of Soil Science and Plant Nutrition, which was submitted to.

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Abstract

Purpose: In Peru, quinoa is cultivated in agricultural systems exposed to abiotic stresses. Selenium biofortification enriches crops and enhances their defense system against water deficit. This study aimed to determine the effect of soil Se application for enhancing Se content in quinoa grains and plant tolerance under water deficit.
Methods: Selenium rates were applied at 0, 0.25, 0.5, 1.0, and 2.0 mg kg^{-1}, under irrigated and water-deficit conditions. Gas exchange, SPAD index, Se content, and proline were quantified in leaves. Dry mass was recorded in grains and shoots, whereas Se content, free amino acids, total proteins, Se and macronutrient accumulation, and Se-recovery were quantified only in grains.
Results: The water deficit diminished the transpiration rate and grain dry mass, but it increased SPAD index. The grain dry mass was increased at 0.25 and 0.5 mg kg^{-1} Se whereas it was reduced at 1.0 and 2.0 mg kg^{-1} Se. Selenium content in grains and leaves and Se accumulation by grains increased concerning Se rates in soil water conditions. Under water deficit, the best photosynthetic rate, instantaneous carboxylation efficiency, water use efficiency, shoot dry mass, and total proteins were at 0.5 mg kg^{-1} Se. In grains, free amino acids and N, P, and K accumulations were maximum at 2 and 0.25 mg kg^{-1} Se, respectively.
Conclusion: Selenium biofortification enriched quinoa grains with selenium. The antioxidant non-enzymatic activity might be one of water deficit tolerance mechanisms promoted by Se on quinoa var INIA Salcedo and would be enhanced mainly at 0.5 mg kg^{-1} Se.

Keywords Biofortification, *Chenopodium quinoa*, Drought mitigation, Plants' defense system, Antioxidant non-enzymatic activity

1 Introduction

Selenium (Se) is one of the essential micronutrients for humans and animals and has a key role in the functioning of biological processes. Selenium is present in the active centers of

antioxidant enzymes, which in turn maintain the integrity of components and membranes cell. Likewise, Se reduces the risk of cardiovascular diseases and cancer, promotes proper thyroid hormone metabolism, improves mental disorders, and strengthens the immune system (Hossain et al. 2021; Kieliszek 2019; Sarwar et al. 2020). Although Se is associated with relevant biological processes in animals, many staple foods worldwide contain insufficient Se levels for the human diet. Low Se intake causes human health disorders such as chronic degenerative diseases (Das et al. 2017; Hossain et al. 2021; Kieliszek 2019; Lopes et al. 2017). Thus, the development of strategies to increase the level of Se in the diet is crucial to avoid serious human illnesses. For instance, biofortification represents a sustainable strategy to enhance the nutritional quality of plant-based foods (Hossain et al. 2021).

In the field, the agronomic biofortification is achieved increasing the Se bioavailability for crops growing on Se-deficient soils. Biofortification has been successfully used to ensure adequate Se intake to reduce Se deficiency in humans. This is particularly important since plants are one of the main sources of available Se, i.e., Se in plants tends to be present in organic forms, which have greater bioaccessibility (Hossain et al. 2021; Raina et al. 2021; Sarwar et al. 2020; Zhou et al. 2021). Research shows that this strategy enriches a wide range of crops grown in Se-deficient soils, such as rice (Andrade et al. 2018; Lessa et al. 2019), wheat (Lara et al. 2019), potato (de Oliveira et al. 2019), common bean (Vega-Ravello et al. 2022), coffee (Mateus et al. 2021), radish (Cipriano et al. 2022b), sorghum (Cipriano et al. 2022a), and others. Moreover, selenate represents the best source for biofortification, mainly when Se is applied in oxidic soils due to its higher availability compared with selenite (Ahmad et al. 2021; Araujo et al. 2020; Cipriano et al. 2022b; dos Santos et al. 2022; Ekanayake et al. 2015).

Overall, the lack of water affects crop productivity by reducing photosynthesis and plant growth. It has become a latent threat to food security since it alters the food chain. Research in crop management has produced new technologies to deal with the low water availability; such technologies have been developed based on the study of the plant water tolerance mechanisms. One of the most widely used ways to cope with this adverse condition is enhancing the plant defense system through the activation of the enzymatic and non-enzymatic antioxidant systems (Farooq et al. 2012; Hussain et al. 2019; Nahar et al. 2016; Sharma et al. 2019).

Although Se is not an essential element for higher plants, Se is beneficial to plants's physiological and metabolic functions. For instance, Se can trigger the plant's defense system and stimulate the tolerance level of plants to abiotic stress such as drought. Likewise, Se decreases water loss, regulates photosynthesis, and improves biomass production through activities of antioxidant enzymes and non-enzymatic antioxidants. These reduce the production

and accumulation of reactive oxygen species (ROS) (Adnan 2020; Chauhan et al. 2019; Hawrylak-Nowak et al. 2018; Hossain et al. 2021; Lanza and Reis 2021; Sarwar et al. 2020). Several studies on water deficit have demonstrated that there is a positive effect of Se on crops such as olive (Proietti et al. 2013), wheat (Nawaz et al. 2017), maize (Bocchini et al. 2018), rice (Andrade et al. 2018), sweet potato (Huang et al. 2020), common bean (Vega-Ravello et al. 2022), among others.

Quinoa is a grain with high nutritional value due to its quality and protein content, even higher than that of many cereals. It was the main food of ancient cultures of the Andes. This Andean superfood with many health benefits has conquered the world market, and greater production areas and higher yields are expected to take place to supply the growing demand (FAO 2011; Filho et al. 2017; Jacobsen et al. 2003b; Patel 2015). In addition, the genetic diversity of the crop and its great adaptability to adverse conditions have allowed its production in several ecosystems (FAO 2011; Hinojosa et al. 2018; Jacobsen et al. 2003a; Saddiq et al. 2021). This quinoa's growth plasticity and the nutritional quality of its grains are potential characteristics to sustain food security worldwide; thus Food and Agricultural Organization (FAO) declared 2013 as the "International year of quinoa" (Patel 2015). On the other hand, in 2005, the Peruvian government declared several Andean crops, including quinoa, as National Natural Heritage, promoting their genetic material conservation and production chain activities (El Peruano 2005). Additionally, international interest in this natural food of high nutritional value encouraged Peru to establish on June 30th of every year as the "National Day of Andean grains", which also includes amaranth, cañihua, and tarwi (El Peruano 2013), as previously reported by Jacobsen et al. (2003b). Peru is the world leader in quinoa production and exports (FAOSTAT 2022). Quinoa cultivation is mechanized and has surface irrigation (Gómez and Aguilar 2016); however, it should still improve because the lack of water on the Peruvian coast is the main constraint for agricultural production (INEI 2013). Therefore, some lots are shifting to drip irrigation. Thus, government policies should be addressed towards implementing technical irrigation and other technologies to increase water use efficiency or plant tolerance to water deficit. In this context, the addition of Se could be one possibility for mitigating the water deficit in the region.

Research on Se content in quinoa grains is not widely performed. One study reported values of Se below the detection limit (Nascimento et al. 2014), 0.039 (Ruales and Nair 1993), and 33 mg Se per kg dry mass (Al-anbari and Al-taweel 2019) in quinoa grains; the first two are considered low. To the best of our knowledge, there are no previous studies on Se application

for biofortifying quinoa grains nor assessing how Se in the plant may contribute to its development in low soil water availability conditions.

Given the nutritional importance of the balanced supply of all essential amino acids by quinoa grains for humans and the water resource limitation for its production in Peru, we developed the present study to Se-enrich quinoa grains, food with high nutritional properties, and improve the quinoa tolerance to water deficit, mainly.

We stated that soil Se application enhances the quality nutrition of quinoa grains and the quinoa crop's capacity to tolerate water deficit. In addition, our results will contribute to improving population health in Peru, at some extent. The agronomic biofortification strategy has been successful reducing the high anemia rate in children under three years old through the iron genetic biofortification of the potato, one of the most widely consumed staple crops (PMA 2010). However, to the best of our knowledge, there are no studies evaluating the agronomic biofortification in quinoa. In this context, our study aims to increase Se content in Andean grains widely consumed in Peru to contribute to the struggle against hidden hunger and determine Se's physiological and metabolic benefits on quinoa crop under water deficit, even more when water shortage is a latent threat to food security.

2 Material and methods

2.1 Soil description and experimental design

The pot study was carried out in a greenhouse located at Soil Science Department, Universidad Nacional Agraria La Molina - UNALM, La Molina, Lima, Peru. The soil was collected from the 0–20 cm depth in an Entisol profile from alluvial plain located on the campus of UNALM (12° 04´ 54.5´´ S latitude, 76° 57´ 08.3´´ W longitude, 239 m above sea level), air-dried, and sieved through a 2-mm mesh. Next, a composite soil sample was analyzed following methodologies suggested by Ministry of Agriculture and Irrigation (MINAGRI) of Peru (Bazán 2017) and soil properties are shown in Table 1. Total soil Se content was 0.26 mg kg^{-1} and was determined following to the 3051A method of the United State Environmental Protection Agency (USEPA 2007).

Table 1 Soil physical and chemical properties

pH$_{(1:1 \text{ in water})}$	EC$_{(1:1)}$ (dS m^{-1})	SOM[a] (%)	P[b] (mg kg^{-1})	K[b]	CEC[c] (cmol$_c$ kg^{-1})	Ca[d]	Mg[d]	K[d]	Na[d]	Clay (%)	Silt	Sand
8.09	0.83	1.15	21.4	379	14.88	12.06	1.57	0.74	0.51	32	30	38

[a]SOM=soil organic matter, [b]available values, [c]CEC=cation exchange capacity, [d]exchangeable values

Pots were filled with 7 kg of soil. Fertilization for N, P and K followed the recommendation of Malavolta (Malavolta 1981), being applied 210, 200, and 150 mg kg^{-1} of N, P, and K, respectively. Nitrogen and K rates were split to three and two applications, respectively, and P rate was added at once before transplanting. Selenium rates - 0.00, 0.25, 0.50, 1.00, and 2.00 mg kg^{-1} – at two soil water conditions (irrigated and water deficit) were applied in the soil as sodium selenate (Na$_2$SeO$_4$, Sigma-Aldrich, Saint Louis, MO, USA) after transplanting. Therefore, the experiment was set up as a completely randomized design in a 5×2 factorial scheme (5 Se rates and 2 soil water conditions) with four replicates, totaling 40 experimental plots.

Chenopodium quinoa W. var INIA Salcedo seeds, an early Peruvian variety adapted to the Altiplano, inter-Andean valleys, and coast, were sown seedling tray and then transplanted to each pot. Six seeds were sown per cell and thinned ten days later at one seedling in each cell.

2.2 Establishment of water deficit

Germination-emergence and grain-filling stages are critical periods for water deficit because of determining productivity (Gómez and Aguilar 2016). All pots were irrigated to reach soil moisture close to water holding capacity (WHC) from the beginning of the experiment until the grain-filling stage began. Therefore, water deficit treatments were differentiated at 59 days after transplanting (DAT), when the quinoa plants started the grain-filling stage. These treatments were subjected to water deficit for five days, where water deficit was assessed by the fraction of transpirable soil water (FTSW) following procedure carried and described by Vega-Ravello et al. (2022). Then, water deficit treatments were re-irrigated to WHC.

2.3 Gas exchange and SPAD chlorophyll index measurements

Gas exchange and SPAD chlorophyll index were assessed on the last day of the water deficit (64 DAT) between 8:00 and 11:00 a.m. on the youngest fully expanded leaf. A portable photosynthesis system (CIRAS-3, PPSystem, Hitchin, UK) was used to measure stomatal conductance (g_s - mol H$_2$O m^{-2} s^{-1}), transpiration rate (E - mmol H$_2$O m^{-2} s^{-1}), and photosynthetic rate (A - μmol CO$_2$ m^{-2} s^{-1}). Instantaneous carboxylation efficiency (E_iC - mol air^{-1}) and water use efficiency (WUE - μmol CO$_2$ mmol^{-1} H$_2$O) were estimated through to ratio

A/C_i (internal CO$_2$ concentration in the substomatal chamber) and A/E, respectively. CIRAS-3 was configured to density of the photosynthetically active photon flux of 1000 μmol m^{-2} s^{-1}, vapor pressure deficit of 2.66 kPa, pump flow of 500 μmol s^{-1}, and the block temperature of 25°C.

The SPAD chlorophyll index was assessed using a portable chlorophyll meter (SPAD-502, Konica-Minolta, Japan). The value recorded was the average of three regions of each leaf (basal, medium, and apical region). Also, in each leaf region, three measures were obtained to calculate the average value per region.

2.4 Biometric measurements and chemical analysis

Quinoa (*C. quinoa*) plants were grown during 150 days and at the end of harvest maturity with a grain moisture content of ~12-14% (Gómez and Aguilar 2016), plant shoots were harvested and separated into leaves, stems, and grains, which were dried in a forced-air oven at 70°C for 48 h. Then, dry mass of leaves and grains were milled to be digested in nitric acid according to the USEPA 3051A method (USEPA 2007), with focus to determine P, K, and Se contents. Phosphorus and K contents were measured by ICP-OES, whereas Se content was determined by atomic absorption spectrometry with graphite furnace atomization (GFAAS) (AAnalyst™ 800 AAS, Perkin Elmer). On the other hand, nitrogen content was determined by digestion with sulfuric acid and titration by the Kjeldahl method (Malavolta et al. 1997). The QA/QC protocol for Se analyses was assured with the addition of two standard/certified reference materials - White Clover-BCR402 (Institute for Reference Materials and Measurements - IRMM, Geel, Belgium) and Peach Leaves-SRM 1547 (National Institute of Standards & Technology - NIST, Gaithersburg, MD, USA) - in each batch of digestion, with Se recovery values being close to 87%.

Nutrient accumulation in grains is expressed as the total amount of nutrient found in grains and was obtained by multiplying the nutrient content and the dry mass of grains. Se-recovery is Se use efficiency by quinoa grain. It was calculated as the percentual relationship between the amount of Se accumulated by grains and the amount of Se applied to the soil of the same treatment (Vega-Ravello et al. 2022).

2.5 Biochemical analysis

Dry mass of quinoa grains and leaves was used for biochemical analysis. Proline content was analyzed in leaves, and free amino acid and total protein contents were quantified in grains.

Proline was extracted from leaves, 0.20 g of ground dry mass was weighed, and 10 mL of 3% sulphosalicylic acid was added. First, the mixture was shaken with a VORTEX and then with a rotary shaker at 160 RPM for one hour, centrifuged at 5000 RPM for 10 min, and the supernatant was collected to quantify proline content (Bates et al. 1973).

For extraction of amino acids and proteins, 0.20 g of ground dry mass of grains was homogenized in 10 mL of potassium phosphate at pH 7.8 (extraction buffer solution), shaken with a VORTEX, placed at 40°C water bath for 30 min, centrifuged at 5000 RPM for 10 min, and the supernatant was collected for further assessments. Aliquots from extracted solution were used to determine free amino acids (Yemm and Cocking 1955) and total proteins (Bradford 1976).

2.6 Statistical analysis and data processing

Results were analyzed using programming language R (R Core Team 2022). Statistical differences were determined by analysis of variance (ANOVA), and treatment means were compared by Tukey's test at 5% probability. Also, the combined effect of treatments on variables studied was evaluated through principal component analysis (PCA) of individuals. The graphics were created, and regression models were fitted using the SigmaPlot for Windows software, version 11.0 (Systat Software Chicago, IL, USA).

3 Results

3.1 Gas exchange and SPAD chlorophyll index

Water deficit significantly modified ($p \leq 0.05$) gas exchange behavior, irrespectively of soil Se addition. More specifically, water deficit reduced stomatal conductance (g_s), transpiration rate (E), photosynthetic rate (A), instantaneous carboxylation efficiency (E_iC), and water use efficiency (WUE) (Fig. 1a-e) and increased SPAD chlorophyll index (Fig. 1f).

Under water deficit, Se application did not improve g_s; however, a slight tendency to increase was observed at a rate of 0.5 mg kg^{-1} Se (Fig. 1a). Photosynthetic rate and E_iC were enhanced by Se supply and were maximum at this same rate (Fig. 1c-d). Likewise, rates of 0.5 and 1 mg kg^{-1} Se showed the highest WUE; nevertheless, only when Se was added at 1 mg kg^{-}

[1] the *WUE* under water deficit was higher by 57% compared to its irrigated treatment (Fig. 1e). On the other hand, *E* drastically reduced by 69% (Fig. 1b) and the SPAD chlorophyll index increased by 7% (Fig. 1f) due to the lack of water, both in relation to the irrigated condition.
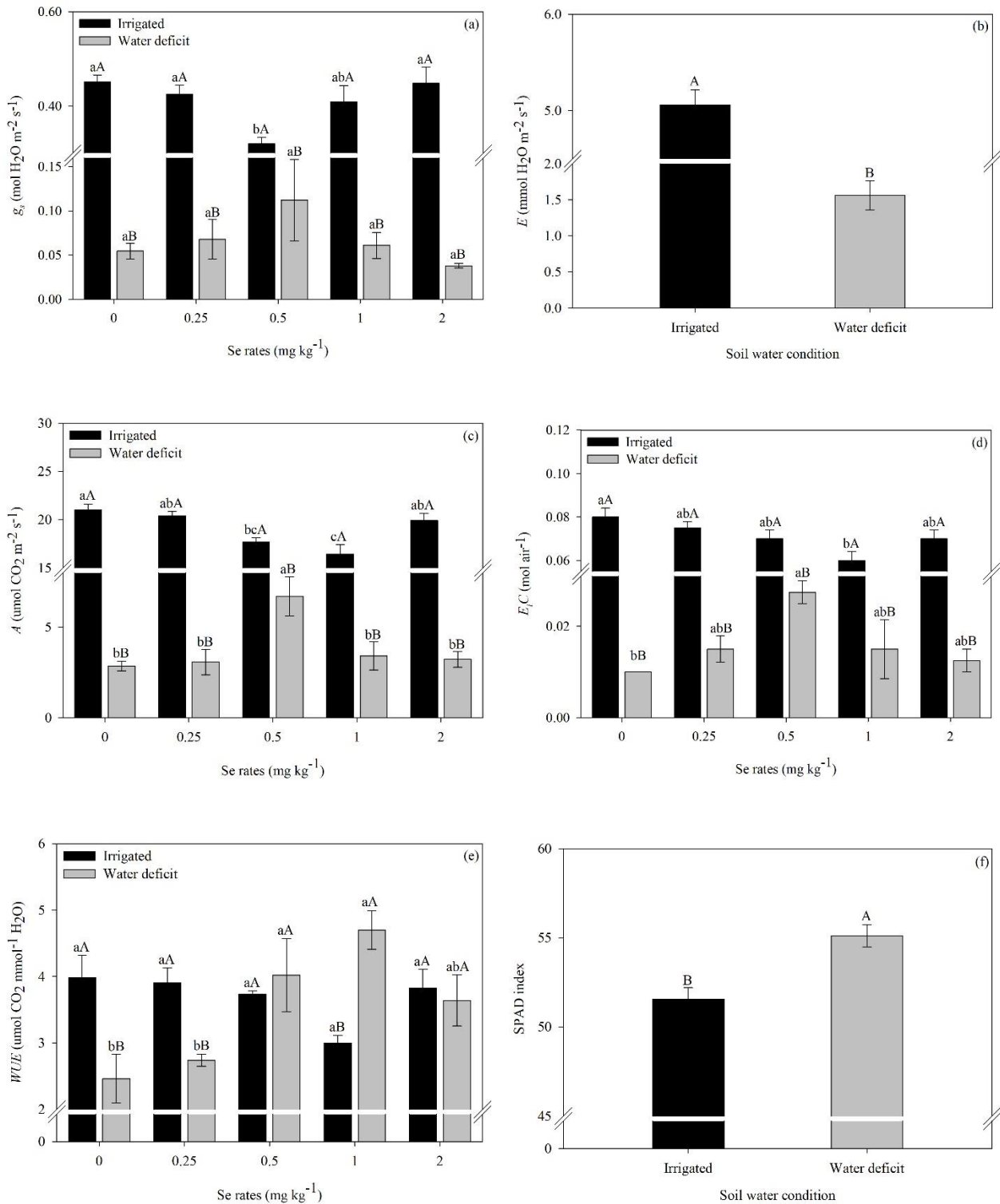
Fig. 1 Stomatal conductance - g_s (a), transpiration rate - *E* (b), photosynthetic rate - *A* (c), instantaneous carboxylation efficiency - E_iC (d), water use efficiency - *WUE* (e), and the SPAD index (f) in leaves of quinoa after water deficit. Uppercase letters compare the means of soil water conditions at each Se rate,
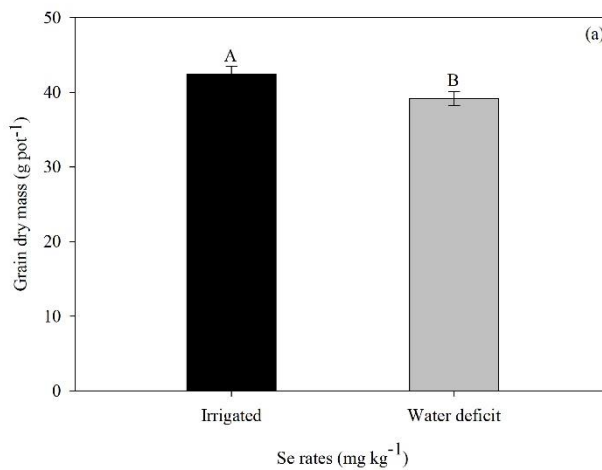
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and lowercase letters compare the means of Se rates at each soil water condition, according to Tukey's test (at 5% probability). Vertical bars indicate standard errors of average values: (a), (c), (d), and (e) n = 4, and (b) and (f) n = 20
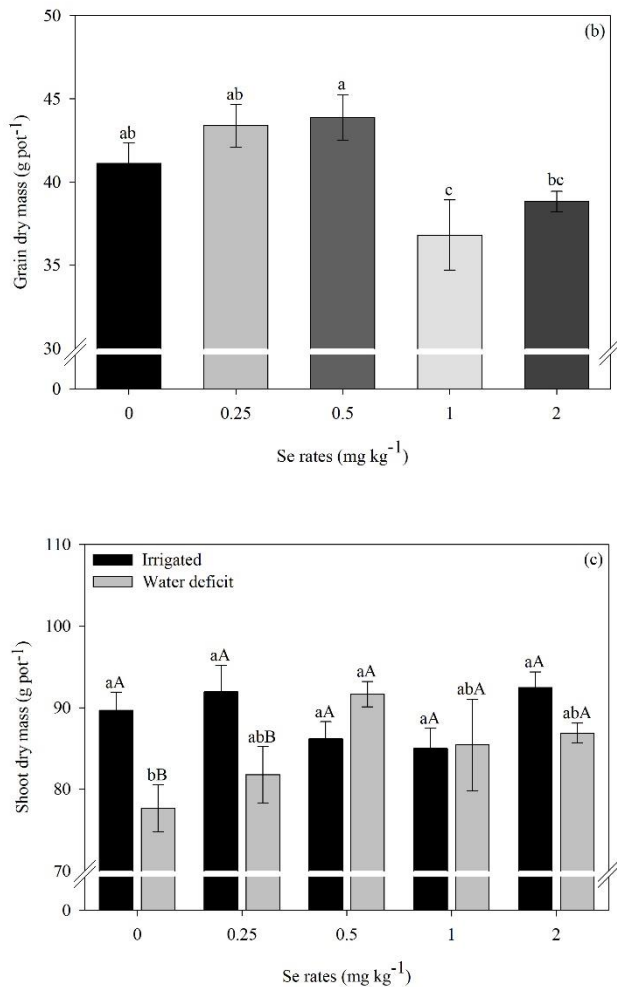
3.2 Production of dry mass

Grain dry mass production changed due to the studied factors (Se rates and soil water conditions), however, there was no significance for the interaction between those two factors ($p \leq 0.05$) (Fig. 2a-b). Conversely, shoot dry mass was affected ($p \leq 0.05$) by the interaction of these factors (Fig. 2c).

The water deficit reduced grain dry mass by 8% with respect to the irrigated condition (Fig. 2a). At Se rates, grain dry mass production tended to increase at 0.25 mg kg^{-1} and was maximum at 0.5 mg kg^{-1}. Conversely, at Se rates of 1 and 2 mg kg^{-1}, grain dry mass reduced by 10 and 6%, respectively, when compared with control (Fig. 2b).

Selenium addition did not influence shoot dry mass production in irrigated treatments. However, under water deficit, shoot dry mass increased when Se was applied at assessed rates, being the maximum value of shoot dry mass found at 0.5 mg kg^{-1} (Fig. 2c).

Fig. 2 Production of grain dry mass (a and b) and shoot dry mass (c) of quinoa. Uppercase letters compare the means of soil water conditions at each Se rate, and lowercase letters compare the means of Se rates at each soil water condition, according to Tukey's test (at 5% probability). Vertical bars indicate standard errors of average values: (a) n = 20, (b) n = 8, and (c) n = 4
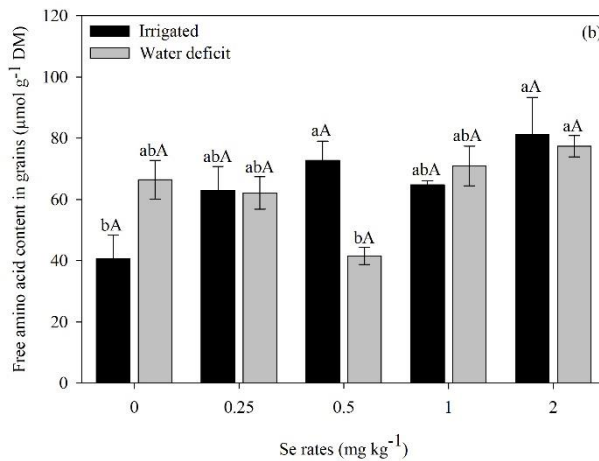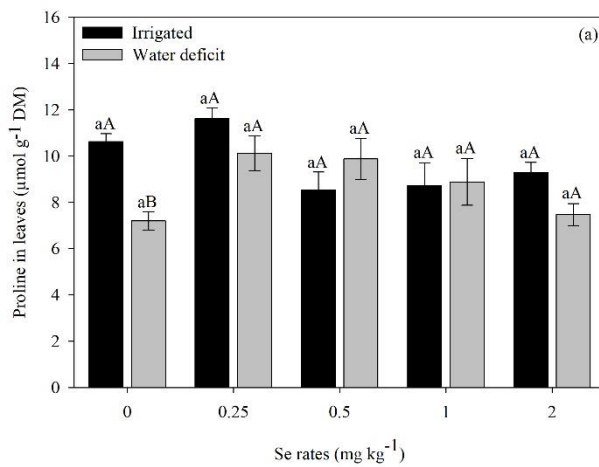
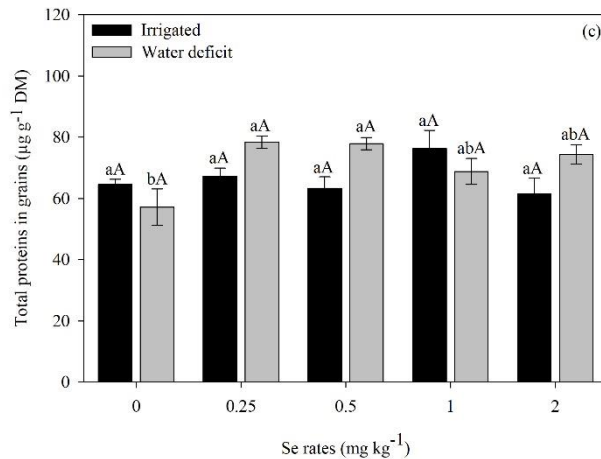### 3.3 Proline content in leaves, free amino acid and total protein contents in grains

Proline content in leaves, free amino acid and total protein contents in grains were significantly affected ($p \leq 0.05$) by the interaction of soil water condition and Se rates (Fig. 3). Leaves from both systems, irrigated and water deficit, exhibited similar proline contents with increasing Se supply. At water deficit, the lowest Se rates (0.25 and 0.5 mg kg^{-1}) tended to maximize the proline content in leaves, which progressively returned to the control level at the highest Se rates (1 and 2 mg kg^{-1}), without showing statistical differences. Comparing soil water conditions within the same Se rate, proline content was different only when Se was not added (control), being 32% lower under water deficit than the value reported to irrigated treatment.

Although the statistic indicated that Se did not support proline increases at water deficit treatments, these proline contents were equal to their irrigated treatments (Fig. 3a).

At irrigated treatments, Se addition enhanced free amino acid content in grains, reaching their maximum values at Se rates of 0.5 and 2 mg kg^{-1} (Fig. 3b); however, it did not modify total protein content in grains (Fig. 3c). Under water deficit treatments, the highest free amino acid content in grains was found at the highest Se rate (2 mg kg^{-1}) (Fig. 3b); while protein formation in grains was favored by Se rates of 0.25 and 0.5 mg kg^{-1}, slightly decreasing at Se rates of 1 and 2 mg kg^{-1} (Fig. 3c).

Fig. 3 Proline content in dry mass (DM) of quinoa leaves (a), free amino acid (b) and total protein (c) contents in dry mass (DM) of quinoa grains. Uppercase letters compare the means of soil water conditions at each Se rate, and lowercase letters compare the means of Se rates at each soil water condition, according to Tukey's test (at 5% probability). Vertical bars indicate standard errors of average values (n = 4)

3.4 Selenium content and accumulation, and Se recovery percentage

Selenium content in grains (Fig. 4a), Se accumulation and Se-recovery by grains (Fig. 5) were significantly affected ($p \leq 0.05$) by the interaction of soil water conditions and Se rates; whereas, Se content in leaves was only influenced ($p \leq 0.05$) by Se rates (Fig. 4b).

Selenium content in grains and leaves (Fig. 4), and Se accumulation by grains (Fig. 5a) were linearly increased upon increasing Se rates in both soil water conditions. In contrast, the percentage of Se-recovery by grains as a function of Se addition followed different patterns depending on soil water conditions. Under irrigated treatments, this variable also increased with the addition of ascending Se rates. However, at water deficit, Se-recovery by grains fitted to a quadratic model. In this case, the maximum value for Se-recovery by grains was obtained with Se addition at 1.44 mg kg^{-1} and was 2%, with this value being decreased afterwards at higher Se rates (Fig. 5b).
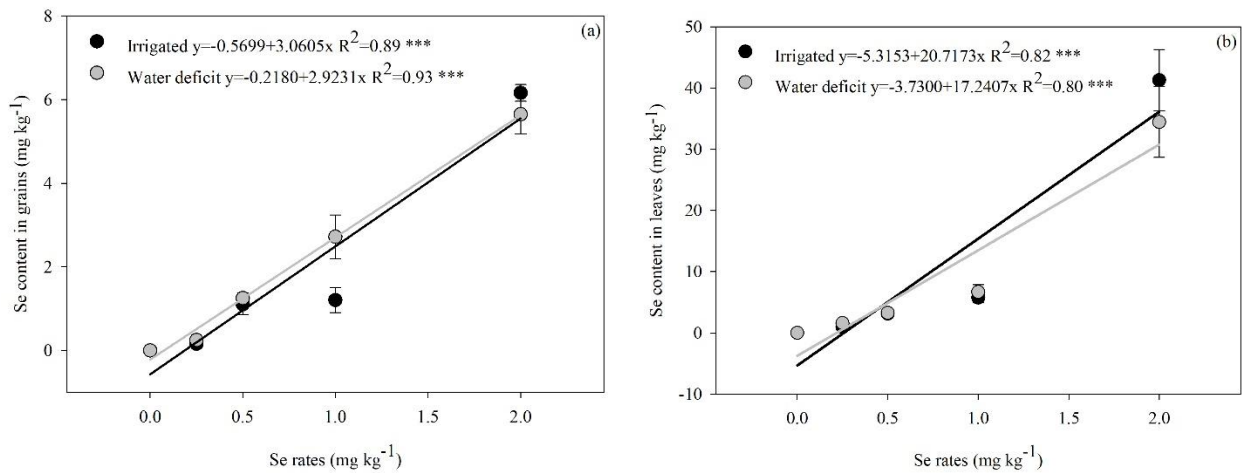
Fig. 4 Selenium content in grains (a) and leaves (b) of quinoa in response to Se rates and soil water conditions. The vertical bars indicate standard errors of average values (n=4). Statistically significant differences are indicated: ***, $p \leq 0.001$
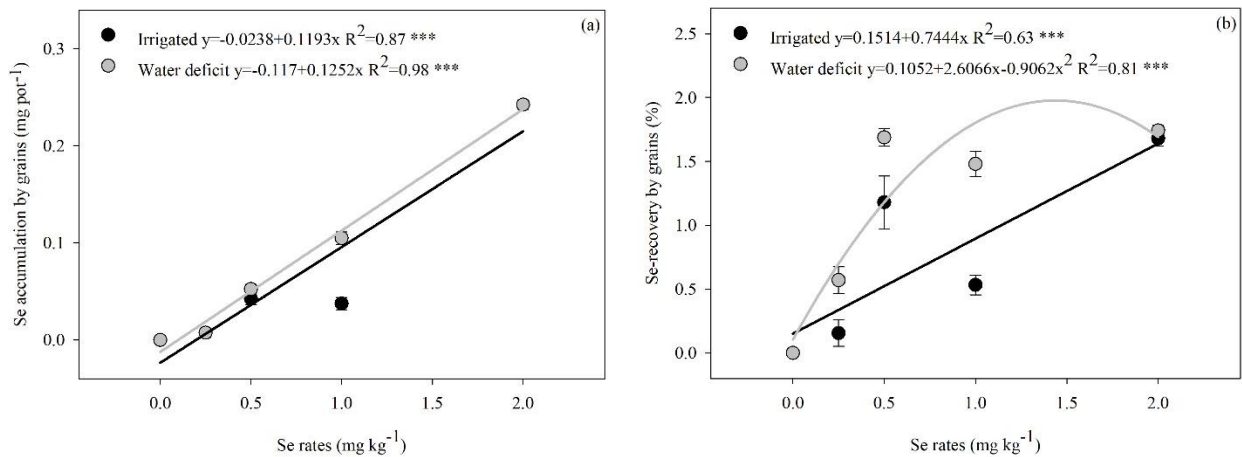
Fig. 5 Selenium accumulation by grains (a) and selenium recovery by grains (b) of quinoa in response to Se rates and soil water conditions. Vertical bars indicate standard errors of average values (n = 4). Statistically significant differences are indicated: ***, $p \leq 0.001$
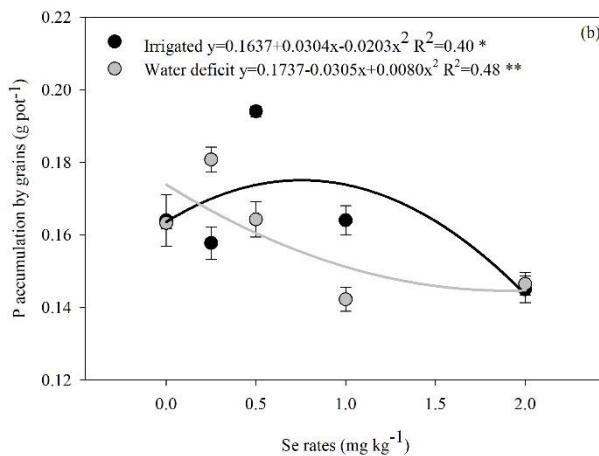
3.5 Nutrient accumulation

Nitrogen, P, and K accumulation by grains was modified ($p \leq 0.05$) by the interaction of Se rates and soil water conditions (Fig. 6). Data of N, P, and K accumulation by grains as a function of Se rates were fitted to quadratic models under the two assessed soil water conditions. As a general trend, in the models for irrigated treatments, the accumulations of these three nutrients increased, reaching their maximum values at rates very close to 1 mg kg^{-1} Se. Selenium applications above these Se rates gradually decreased their nutrient accumulations in

the grain. At the water deficit models, N, P, and K accumulations decreased until values were slightly above 1 mg kg^{-1} Se; after these rates, their accumulations grew (Fig. 6).

Regarding Se addition, quinoa grains in irrigated treatments contained higher amounts of N at 0.25 to 1 mg kg^{-1} Se (Fig. 6a) and of P and K at 0.25 mg kg^{-1} Se (Fig. 6b-c). At the same time, 0.25 mg kg^{-1} Se promoted the highest accumulations of these nutrients in water deficit (Fig. 6).

At water deficit treatments, when their controls were compared with the irrigated treatment controls, accumulation of N was 20% higher (Fig. 6a), P was similar (Fig. 6b), K was 15% lower (Fig. 6c). Nitrogen and K accumulation maintained the levels of their irrigated treatments at 0.25 and 2 mg kg^{-1} Se (Fig. 6a and 6c). Nitrogen at Se rates of 0.5 and 1 mg kg^{-1} decreased 12 and 18%, respectively, compared with their irrigated treatments (Fig. 6a), while K had more drastic drops, 36 and 29%, respectively (Fig. 6c). Concerning P accumulation, it was 15% higher, 15 and 13% lower, and similar to their irrigated treatments at Se rates of 0.25, 0.5, 1, and 2 mg kg^{-1}, respectively (Fig. 6b).

(a) Irrigated $y=1.0995+0.4989x-0.2560x^2$ $R^2=0.62$ ***
Water deficit $y=1.3253-0.3680x+0.1405x^2$ $R^2=0.42$ *

(b) Irrigated $y=0.1637+0.0304x-0.0203x^2$ $R^2=0.40$ *
Water deficit $y=0.1737-0.0305x+0.0080x^2$ $R^2=0.48$ **

Fig. 6 Nitrogen accumulation (a), phosphorus accumulation (b), and potassium accumulation (c) by quinoa grains in response to Se rates and soil water conditions. Vertical bars indicate standard errors of average values (n = 4). Statistically significant differences are indicated: ns, *, **, ***, $p \leq 0.001$
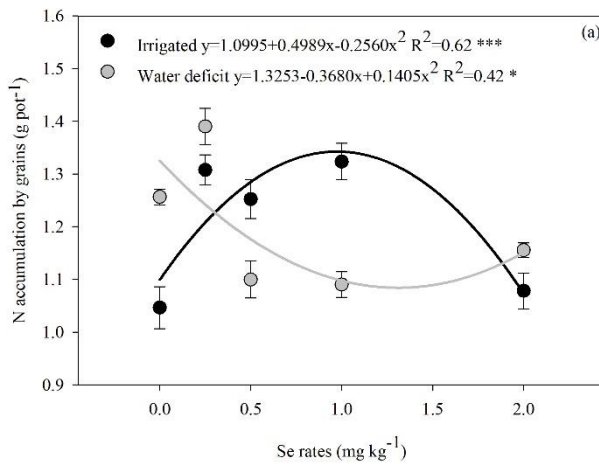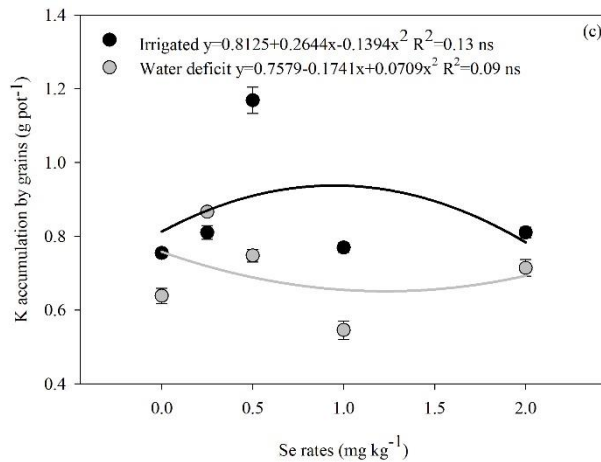
3.6 Principal component analysis (PCA) of individuals

Principal component analysis (PCA) for individual revealed that two principal components were representative of the interaction among evaluated variables and accounted for 54.6% of the total variance (Fig. 7). The first component (Dim1) explained 30.7% variation, while the second component (Dim2) explained 23.9%. In Dim1 ($|cor|>0.5$), major variance was contributed by A, g_s, E, E_iC, grain dry mass, Se content in grains and leaves, Se accumulation and Se-recovery by grains, P and K accumulation by grains, and proline content in leaves. In Dim 2 ($|cor|>0.5$), major variance was contributed by A, g_s, E, WUE, E_iC, shoot dry mass, Se content in grains and leaves, and Se and N accumulation by grains.

The effect of treatments on variables distinguished six groups by confidence regions at a 95% confidence level. At the same time, the distance among the ellipses allowed the identification of two completely different groups among the irrigated treatments (from I-0 to I-1 and I-2 mg kg^{-1} Se) and two more groups among the water deficit treatments (from WD-0 to WD-0.25 and from WD-1 to WD-2 mg kg^{-1} Se). On the other hand, the interception of the ellipses indicated similarity in the behavior of the water deficit treatment at 0.5 mg kg^{-1} Se with the groups composed by the irrigated treatments from 0 to 1 mg kg^{-1} Se and water deficit treatments from 0 to 0.25 and from 1 to 2 mg kg^{-1} Se. Also, irrigated treatment at 1 mg kg^{-1} Se exhibited the same behavior, except with the water deficit treatment at 2 mg kg^{-1} Se because they are mutually exclusive.
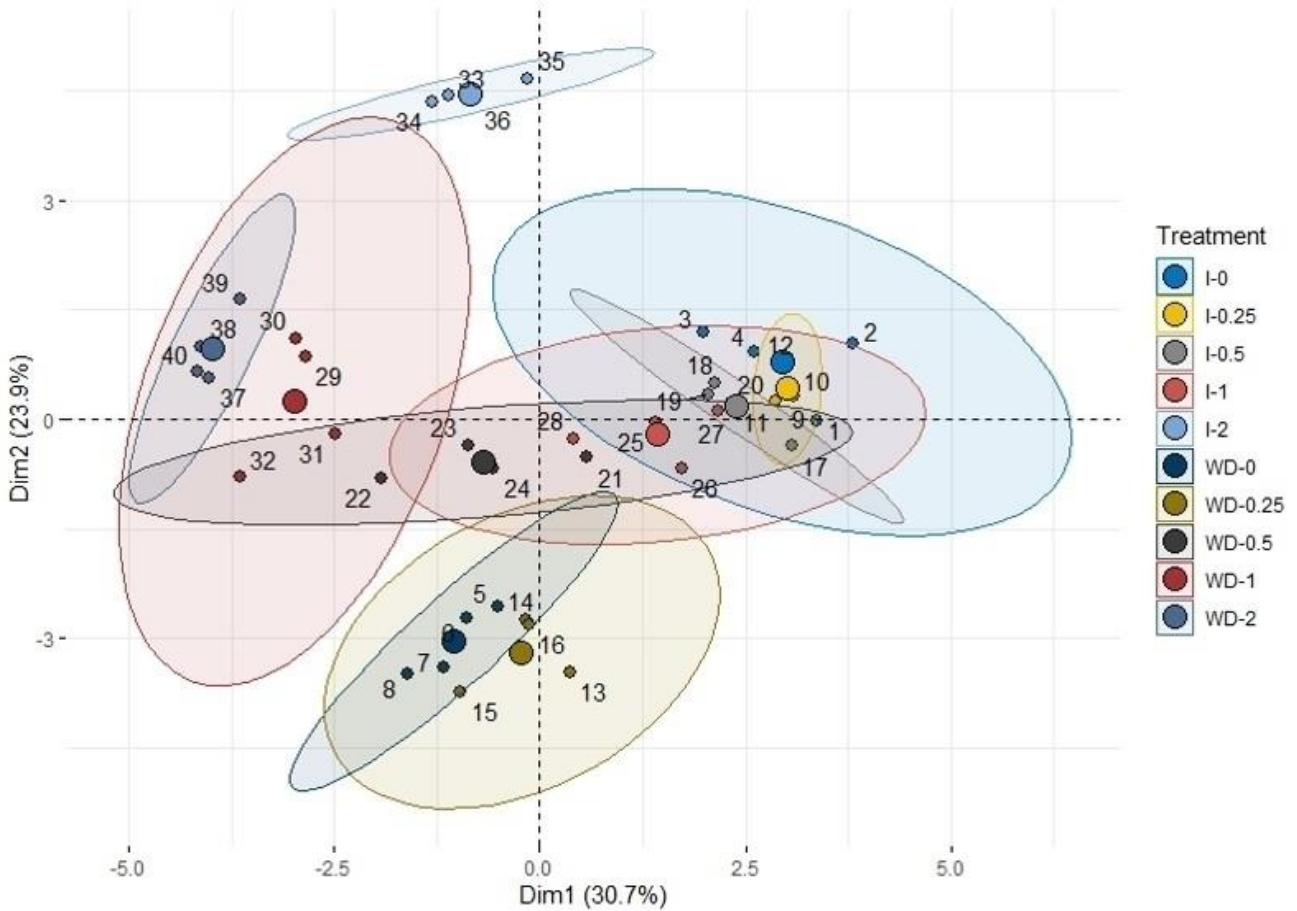
Fig. 7 PCA graph of individuals. Circles of the same color represent the treatments of soil water conditions (irrigated (I) and water deficit (WD)) in each Se rate (0.00, 0.25, 0.50, 1.00, and 2.00 mg kg⁻¹)

4   Discussions

The tissue elasticity and growth plasticity of quinoa permit it to tolerate water deficit (Hinojosa et al. 2018). In our study, the decrease in g_s (Fig. 1a), E (Fig. 1b), A (Fig. 1c), and EiC (Fig. 1d) reflected moderate (0.5 mg kg⁻¹ Se) and severe (0, 0.25, 1, and 2 mg kg⁻¹ Se) water deficits (Flexas and Medrano 2002) and the sensitivity of quinoa leaf gas exchange to water deficit which suggests an accumulation of abscisic acid (ABA) as a promoter to rapid stomata closure (Jacobsen et al. 2009). This is a feature of isohydric properties of quinoa used to maintain its leaf hydric potential (Killi and Haworth 2017). Likewise, inhibition of photosynthesis expressed in the drop of A (Fig. 1c) and lower shoot dry mass formation (Fig. 2c) at low water availability involved the reduction of WUE (Fig. 1e) since moderate and severe water deficits obtained have reduced the contribution of photosynthesis and photorespiration, as indicated by Flexas and Medrano (2002).

Despite this adverse condition, the beneficial effects of the presence of low Se rates play important roles on the efficiency of the plant's photosynthetic metabolism since Se act as an antioxidant (Adnan 2020; Hawrylak-Nowak et al. 2018; Lanza and Reis 2021; Raina et al. 2021; Sarwar et al. 2020), in quinoa plants (Khalofah et al. 2021). Our results have shown that low Se rates (0.25 and 0.5 mg kg^{-1}) regulated quinoa photosynthesis under water deficit to mitigate the impact of fixing less carbon. Carbon dioxide input tended to increase in quinoa leaves, and g_s reached its maximum concentration at 0.5 mg kg^{-1} Se (Fig. 1a) since this Se rate enhanced its water deficit status, turning it into a moderate water deficit. This suggested that higher carbon was available, and *EiC* (Fig. 1d) was greater to enhance *A* (Fig. 1c) by increasing chlorophyll, as pointed out by Khalofah et al. (2021). In contrast, higher Se rates (1 and 2 mg kg^{-1}) modified the beneficial trend of lower Se rates since it behaved as a pro-oxidant, as referred Khalofah et al. (2021). The toxic Se effect could be increasing the oxidative stress that altered the physiological and metabolic processes in the plant, such as the gas exchange reduction.

In addition, Se rate of 0.5 mg kg^{-1} enhanced *WUE* (Fig. 1e) since the higher photosynthetic activity increased shoot dry mass production (Fig. 2c) with lower water availability. This influence extends at the Se rate of 1 mg kg^{-1}, where *WUE* is maximum. Although the shoot dry mass produced is slightly lower at this rate (Fig. 2c), the biomass per unit of water is higher. This behavior is similar to that of the genotypes tolerant to water deficit (Saddiq et al. 2021).

Water deficit increases oxidative stress which affects chlorophyll synthesis (Farooqi et al. 2020; Hussain et al. 2019). In contrast to our expectations, quinoa leaves under water deficit exhibited better SPAD chlorophyll index status, which could be attributed to a concentration effect (Bocchini et al. 2018) (Fig. 1f).

Quinoa grains accumulated lower dry mass in water deficit (Fig. 2a) than the grains from irrigated treatments. This observation might be in response to reduced photosynthetic activity, which was expressed in low gas exchange. On the other hand, dual role of Se indicated by Khalofah et al. (2021) was observed in the production of grain dry mass and shoot dry mass. The accumulation of grain dry mass in response to Se rates (Fig. 2b) was correlated with the gas exchange; at low Se rates, grain dry mass production was enhanced up to a Se rate of 0.5 mg kg^{-1}; after its accumulation was detrimental. Probably, low Se rates promote activities of antioxidant enzymes and non-enzymatic antioxidants to scavenge ROS and protect the integrity of cell membranes and ensure the continuity of photosynthesis, while high rates contribute to oxidation, as found by Khalofah et al. (2021).

Unlike the behavior of the accumulation of grain dry mass concerning Se rates, the production of shoot dry mass at water deficit increased at all Se rates (Fig. 2c), suggesting that

high Se rates would not be phytotoxic to accumulation of dry mas in quinoa leaves and stems, as reported by Vega-Ravello et al. (2022).

Quinoa is an inherent water deficit tolerant species that has morphological, physiological, and molecular mechanisms to responds against low water availability (Hinojosa et al. 2018). Moreover, proline accumulation is a mechanism that has a dual role as osmoprotectant and an osmolyte (Saddiq et al. 2021). Nevertheless, when comparing irrigated and water-deficient plant controls (Fig. 3a), the proline content found in leaves suggested that quinoa var INIA Salcedo does not use proline accumulation as the primary mechanism of water deficit tolerance, as reported by Hinojosa et al. (2018) for other quinoa varieties. On the other hand, proline content in quinoa leaves in response to soil Se addition did not vary markedly (Fig. 3a), opposite behavior to that found in maize (Bocchini et al. 2018) and in other study involving quinoa plants (Khalofah et al. 2021). Although no notable differences were found, the tendency to accumulate more proline at 0.25 and 0.5 mg kg^{-1} Se under water deficit (Fig. 3a) suggests that lower Se rates promote its increase and other mechanisms to cope with water deficit. Likewise, the increase of proteins by Se rates at water deficit (Fig. 3c) was indicative that proline contributed to maintain their integrity (Chauhan et al. 2019; Hawrylak-Nowak et al. 2018; Hayat et al. 2012; Khalofah et al. 2021; Lanza and Reis 2021; Nahar et al. 2016; Sarwar et al. 2020; Wrobel et al. 2020).

The increase of free amino acid content in quinoa grains in the treatment Se at 2 mg kg^{-1} rate (Fig. 3b), suggests that the Se applied was incorporated by the plant and used in the formation of selenomethionine and selenocystine (Hossain et al. 2021; Sarwar et al. 2020). As we known, amino acids are precursors of proteins which maintain their vital functions in living species. However, under water deficit, the content of free amino acid was not strictly associated to total proteins as amino acids can also act individually as compatible solutes (Farooqi et al. 2020; Hussain et al. 2019). Thus, although the highest amount of amino acids (Fig. 3b) was found at the highest Se rate (2 mg kg^{-1}), the water deficit does not ensure that total amino acids are directed toward protein formation. On the contrary, the lowest Se rates (0.25 and 0.5 mg kg^{-1}) were the most efficient in protecting proteins, which is reflected in their higher amount of total proteins (Fig. 3c).

Even though proline and protein contents did not show notable differences concerning Se rates at water deficit, if there was an increase concerning the control, they could reflect their participation as osmolytes in the osmotic adjustment of quinoa. This behavior could also have enhanced gas exchange by maintaining the cell membranes' integrity.

We observed that the quinoa biofortification has increased in response to the Se application on the soil (Fig. 4). In this study, quinoa was grown in alkaline Peruvian soil (pH = 8.09), which enhanced the availability of selenate in the soil. However, the results obtained from the Se content in grains and leaves (Fig. 4) as well as the Se accumulation in grains (Fig. 5a), and the Se recovery percentage in grains (Fig. 5b) differ from results reported by other authors in similar experiments performed in rice (Andrade et al. 2018), potato (de Oliveira et al. 2019), common bean (Vega-Ravello et al. 2022), and radish (Cipriano et al. 2022b). The content of Se in the quinoa grains var INIA Salcedo were significantly higher than those reported by Nascimento et al. (2014) and Ruales and Nair (1993) and was lower than the study published by Al-anbari and Al-taweel (2019). Still, we must consider that it was a pot experiment. Prauchner (2014) highlighted that Se availability in soil, Se uptake, and the inorganic and organic flow of Se within the plant are influenced by plant species, plant morphology and phenology, chemical Se forms, soil conditions, and crop management. Ahmad et al. (2021) found that selenate was the most efficient inorganic form to increase Se in wheat grains in calcareous soil. Selenate is more water-soluble, predominates in neutral to higher pH soils, and enhances Se uptake and Se translocation using the same sulfate transporters (Hossain et al. 2021; Sarwar et al. 2020; Trippe and Pilon-Smits 2021).

Nitrogen, P, and K accumulations by quinoa grains (Fig. 6) reflected the impact of soil water conditions on plant metabolic and physiologic processes. Water deficit lessened the accumulation of N, P, and K in quinoa grains since low soil water content diminishes root activity and nutrient and water uptake, as reported by Farooqi et al. (2020). In addition, the reduction of gas exchange in leaves intensified oxidative stress in quinoa which disturbed photosynthesis, reducing photosynthate formation and its redistribution to grains.

It is well known that adequate water supply promotes nutrient availability and uptake, and plant growth, while water deficit disturbed them. The lowest Se rate (0.25 mg kg^{-1}) increased the accumulation of N, P, and K in quinoa grains subjected to water deficit (Fig. 6). Selenium promotes antioxidant non-enzyme mechanisms to eliminate free radicals and maintain cell membrane integrity in water deficit by the production of proline and proteins. These mechanisms may explain our findings because we had high levels of proline and proteins under water deficit (Fig. 3a and 3c) as well. The expression of such mechanisms would enhance the photosynthetic process of quinoa. According to Zhou et al. (2020), plants uptake essential nutrients to increase their biomass and take up Se. Also, Schiavon et al. (2020) reported that Se promotes N assimilation in horticultural crops, and Bocchini et al. (2018) referred that Se increases K content in maize due to increased N metabolism.

Groups delimited by the ellipses (Fig. 7) allowed to consolidate the understanding of factor effects under study. Principal component analysis of individuals indicated that most of the variables from irrigated treatments at Se rates of 0, 0.25, 0.5, and 1 mg kg^{-1} (I-0, I-0.25, I-0.5, and I-1) presented similar behaviors. Likewise, water deficit treatment at 0.5 mg kg^{-1} Se (WD-0.5) may be considered as the most favorable rate to mitigate water deficit since its effect on quinoa showed similar behavior than quinoa subjected to irrigated treatments at rates of Se less than 2 mg kg^{-1} (I-2).

5 Conclusions

Our study found that Se biofortification enriches quinoa grains. The antioxidant non-enzyme activity mediated by the accumulation of proline and proteins seems to be one of the mechanisms involved for enhancing the tolerance effect of quinoa var INIA Salcedo. This effect was clearly observed at low rates of Se application under water deficit; the activation of the mechanism mentioned above may allow for regulating gas exchange, provide continuity to photosynthesis, and increase biomass production. The Se rate at 0.5 mg kg^{-1} seems to be the most promising for improving photosynthetic rate (*A*), instantaneous carboxylation efficiency (*EiC*), and shoot dry mass, whereas the addition of 0.25 mg kg^{-1} Se has shown to be favorable for increasing the accumulation of N, P, and K in grains.

Author Contribution R Vega-Ravello, conceptualization, methodology, validation, formal analysis, investigation, data curation, writing – original draft, writing – review & editing. M Romero-Poma, methodology, validation, formal analysis, investigation, writing – original draft. C de Oliveira, supervision, writing – review & editing. L Guilherme, resources, supervision, writing – review & editing. G Lopes was the project advisor, planned and supervised all phases of this research. Funding acquisition.

Declarations

Conflicts of interest The authors declare that they have no conflicts of interest.

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ARTICLE 3: SOIL SELENIUM BIOFORTIFICATION TO ENHANCE NUTRITIONAL AND PHYSIOLOGICAL RESPONSES OF QUINOA CROP GROWN UNDER WATER DEFICIT IN AN OXISOL

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Highlights

- Selenium biofortification enriched quinoa grains with Se.
- Selenium activated antioxidant defense systems to alleviate water deficit.
- The cultivar BRS Piabiru expressed its mechanism of tolerance to water deficit.
- The cultivar BRS Piabiru has the potential to contribute to sustainable food security.

ABSTRACT

Some grains consumed in Brazil have low selenium (Se) content since they are cultivated in regions with Se-deficient soils and are also affected by abiotic stress such as water deficit. This research aims to enhance the nutritional quality of quinoa grains with selenium by soil Se application and to elucidate the Se-promoted mechanisms associated with tolerance to water deficit. Selenium was soil applied at two water conditions - irrigated and water deficit - at 0, 0.125, 0.25, 0.5, 0.75, 1.0, and 2.0 mg kg^{-1} rate. Gas exchange, SPAD index, SOD, CAT, APX activities, and hydrogen peroxide concentration in leaves were evaluated at the end of the water deficit. We also quantified dry mass in shoots and grains, Se content in grains and leaves, free amino acid and total protein content in grains, Se and macronutrient accumulation by grains, and Se-recovery by grains at harvest. Selenium content in grains and leaves, and Se accumulation by grains increased with Se rates at both water conditions, and Se-recovery by grains increased at diminishing rates. The water deficit lessened gas exchange (g_s, A, and E), E_iC, and SPAD index; however, Se rates up to 1 mg kg^{-1} promoted their increase. Under water deficit, Se rates enhanced WUE; SOD, CAT, and APX enzyme activity; and free amino acid content, whereas reduced H_2O_2 concentration; grain dry mass; N, P, K, and Mg accumulation

at 0.75 mg kg^{-1}, and shoot dry mass at 0.75 and 1 mg kg^{-1}. Soil selenium biofortification enhanced quinoa grains' nutritional quality with selenium and antioxidant enzyme activity. Selenium also promoted free amino acid accumulation, which in turn enhanced the tolerance to water deficit. The integrated action of these defense mechanisms mitigated severe oxidative stress, thus regulating gas exchange that allowed the continuity of the photosynthetic process of quinoa cultivar BRS Piabiru, particularly at 0.75 mg kg^{-1}.

Keywords: Chenopodium quinoa, Antioxidant enzymatic activity, Antioxidant non-enzymatic activity, Gas exchange, Tolerance mechanisms, Mitigation

1. Introduction

Staple foods that are cultivated in soils with low mineral availability tend to produce nutritional deficiencies in consumers. Many soils from different regions of Brazil are considered a selenium-deficient soil (Carvalho et al., 2019; dos Reis et al., 2017; Lanza and Reis, 2021; Schiavon et al., 2020), and consequently, crops produced in them have low Se contents (dos Reis et al., 2017). Conversely, the Brazilian Amazon produces fruits with high selenium content, such as Brazilian nuts. This is due to the soils from Brazilian Amazon exhibiting natural biofortification (dos Reis et al., 2017; Hossain et al., 2021; Silva Junior et al., 2017).

Selenium (Se) is an essential micronutrient for animals and humans. One of its specific roles is involved in the dynamics of the redox process. Selenium is a fundamental part of the active center of antioxidant enzymes, which are crucial for the proper functioning of the human brain and its cardiovascular, immune, endocrine, and reproductive systems. Given the relevant contribution of Se to human health, ensuring an adequate Se-rich diet is essential. The biofortification contributes to enriching Se-deficient soils, which improves Se bioavailability (organic) on crops. Thus, the human diet is highly improved by using biofortification to cultivate staple crops (Hossain et al., 2021; Lanza and Reis, 2021; Sarwar et al., 2020; Schiavon et al., 2020).

Agronomic biofortification is a strategy that improves the quality of food crops by reducing essential micronutrient deficiencies in the human diet (Bouis et al., 2019; Das et al., 2017; Lopes et al., 2017). Studies carried out in Brazilian Se-deficient soils showed a notable increase in Se content and an enhancement in protein quality of most grains produced/consumed across the region, such as rice (Boldrin et al., 2013, 2012; Lessa et al., 2020, 2019), wheat (Lara et al., 2019), soybean (Bamberg et al., 2019), common bean (Vega-Ravello et al., 2022), and sorghum (Cipriano et al., 2022).

In addition to Se biofortification of edible plants reduces the risk of human health disorders. Selenium application generates beneficial and detrimental effects on physiological and metabolic processes in higher plants at low and high concentrations, respectively, as reported in quinoa (Khalofah et al., 2021), despite not fulfilling any essential function (Brown et al., 2021). The protective action of selenium is well recognized in plants that have oxidative stress caused by biotic and/or abiotic factors, such as water deficit (Hossain et al., 2021; Sarwar et al., 2020; Schiavon et al., 2020). This finding is confirmed by Brazilian research on rice (Andrade et al., 2018) and common bean (Vega-Ravello et al., 2022); those studies found that selenium can activate enzymatic antioxidant systems. Thus, selenium becomes an alternative for agricultural activity threatened by water scarcity (United Nations, 2021) in a context in which climate change is intensifying. The water deficit negatively impacts crop production (UNESCO, 2020), impairing plant growth and productivity (Farooqi et al., 2020; Hussain et al., 2019).

Quinoa was one of the main grain crops domesticated and cultivated by the pre-Inca cultures of the Andes for being a highly nutritious food (FAO, 2011; Jacobsen et al., 2003a, 2003b). Grains of quinoa, a pseudo-cereal, have an exceptional balance of essential amino acids, making them superior in quantity and protein quality to those of wheat (Stikic et al., 2012), rice, corn, and bean (Filho et al., 2017; Spehar et al., 2007); however, those grains have low Se content (Nascimento et al., 2014; Ruales and Nair, 1993). Moreover, the genetic diversity of quinoa would contribute to its ability to adapt to marginal soils and tolerate abiotic stresses (Hinojosa et al., 2018; Jacobsen, 2003), such as water deficit (Jacobsen et al., 2009; Killi and Haworth, 2017; Saddiq et al., 2021; Spehar and Santos, 2002). These characteristics describe quinoa as one of the most complete foods capable of playing a key role in the sustainability of food security worldwide and alleviating malnutrition, hence its rising consumption (FAO, 2011; Filho et al., 2017; Jacobsen et al., 2003a), and denominate it as the "golden grain" (Angeli et al., 2020). Besides its multiple uses in food, the utilization of the whole plant in farming systems evoked the interest of Brazil to consider quinoa cultivation as an alternative to diversify its production system in the Cerrado biome. Still, despite efforts to develop research and adapt technologies, its management has not yet been controlled (Spehar et al., 2007).

Quinoa is a crop to face the increasing demand for healthy foods. Given that there is lower water available for agriculture and Brazil's need to diversify its production system, we consider that soil application of Se increases the nutritional richness of quinoa grains as well as the tolerance of this crop to water deficit. Nevertheless, there have not yet been performed studies to biofortify quinoa cultivated in Brazilian soils deficient in Se. Therefore, this research aims

to: i) enrich quinoa grains with Se, improving their nutritional quality to help tackling hidden hunger; and, ii) assess quinoa's tolerance mechanisms promoted by Se to cope with water deficit.

2. Material and methods

2.1. *Soil description, quinoa cultivation and experimental design*

The greenhouse pot experiment was conducted at the Soil Science Department, Federal University of Lavras (UFLA), Lavras, State of Minas Gerais, Brazil. The soil for the study was collected within the first 20 centimeters of an Oxisol profile from a secondary forest located at the UFLA campus, air-dried, sieved through a 4-mm mesh, and analyzed according to the methodologies suggested by the Brazilian Agricultural Research Corporation (EMBRAPA) (Teixeira et al., 2017). The most important soil characteristics were as follows: pH in water = 4.60; soil organic matter = 32.70 g kg^{-1}; available P (Mehlich-1) = 1.18 mg kg^{-1}; available K = 61.90 mg kg^{-1}; exchangeable Ca = 0.45 cmol$_c$ kg^{-1}; exchangeable Mg = 0.28 cmol$_c$ kg^{-1}; exchangeable Al = 1.18 cmol$_c$ kg^{-1}; total acidity (H+Al) = 11.62 cmol$_c$ kg^{-1}; cation exchange capacity (CEC) at pH 7 = 12.51 cmol$_c$ kg^{-1}; clay = 610 g kg^{-1}; silt = 110 g kg^{-1}; sand = 280 g kg^{-1}; and bulk density = 1.03 g cm^{-3}. Total soil Se content was 0.27 mg kg^{-1} and was determined after acid digestion with HNO$_3$ by the 3051A method of the United State Environmental Protection Agency (USEPA, 2007).

Water holding capacity (WHC) was determined before setting up the experiment by weighing some pots after the dry soil was saturated and the excess water drained. Pots were filled with an equivalent weight to 4.2 kg of dry soil. Liming was carried out to raise base saturation to 65%, using limestone (CaCO$_3$/MgCO$_3$ at a ratio of 4:1). Limestone was incorporated and incubated for 50 days while keeping the soil moisture at 50% WHC. The fertilization followed the recommendation of Malavolta (Malavolta, 1981), and 210, 200, 50, 5, 1.5, 0.5, and 0.1 mg kg^{-1} of N, P, K, S, Zn, Cu, B, and Mo were applied, respectively. Nitrogen and K rates were split to three and two applications, respectively. The first N and K fractionation and total P and micronutrient rates were added before transplanting. The second N and K application and the third application of N were performed at 25 and 45 days after transplanting (DAT), respectively. Rates of 0, 0.125, 0.25, 0.5, 0.75, 1, and 2 mg Se kg^{-1} were applied as sodium selenate (Na$_2$SeO$_4$, Sigma-Aldrich, Saint Louis, MO, USA) to soils under irrigated and water deficit treatments after transplanting.

Seeds of *Chenopodium quinoa* W. cultivar BRS Piabiru were provided by EMBRAPA, Brasília, Brazil. This cultivar was the first grain crop recommended for Brazilian systems and originated from an EC 3 breeding line selected from a plant population of Quito, Ecuador (Spehar and Santos, 2002). Four seeds were sown per cell in the seedling tray and thinned twenty days later at one seedling in each cell, then transplanted to each pot when seedlings developed four true leaves.

The experiment was set up as a completely randomized factorial design with 7 selenium rates (0, 0.125, 0.25, 0.5, 1, and 2 mg kg^{-1}) × two soil water conditions (I: irrigated and WD: water deficit) scheme with four replicates, totaling 56 experimental plots.

2.2. *Establishment of water deficit*

The grain-filling stage is one of the quinoa crop's most sensitive phenological stages to water deficit (Spehar and da Silva Rocha, 2010). Since the setting up of the experiment, irrigated and water deficit treatments were watered to WHC. When the grains were filling (39 DAT), water deficit treatments were differentiated by subjecting them to water deficit. The water deficit lasted five days and was evaluated through the fraction of transpirable soil water (FTSW) following the procedure described by Vega-Ravello et al. (2022). Then, water deficit treatments were re-irrigated to WHC.

2.3. *Gas exchange and SPAD chlorophyll index measurements*

Gas exchange and SPAD chlorophyll index were measured on the last day of the water deficit (43 DAT) between 8:00 and 11:00 a.m. on the youngest fully expanded leaf.
A portable infrared gas analyzer (IRGA, model LI-COR 6400, Li-Cor Biosciences, Lincoln, NE, USA) assessed stomatal conductance (g_s - mol H_2O m^{-2} s^{-1}), transpiration rate (E - mmol H_2O m^{-2} s^{-1}), and photosynthetic rate (A - μmol CO_2 m^{-2} s^{-1}). Besides, it calculated instantaneous carboxylation (E_iC - mol air^{-1}) and water use (WUE - μmol CO_2 mmol^{-1} H_2O) efficiencies by ratio A/C_i (internal CO_2 concentration in the substomatal chamber) and A/E, respectively. IRGA was calibrated to density of the photosynthetically active photon flux of 1000 μmol m^{-2} s^{-1}, vapor pressure deficit of 2.66 kPa, pump flow of 500 μmol s^{-1}, and the block temperature of 25°C.

A portable chlorophyll meter (SPAD-502, Konica-Minolta, Japan) evaluated SPAD chlorophyll index. The data reported was the average of three regions of each leaf (basal,

medium, and apical region); moreover, in each leaf region, three readings were carried out to calculate the average value per region.

2.4. Biochemical analysis

A couple of fully expanded fresh leaves were collected to quantify activity of antioxidative enzymes and H_2O_2 concentration after gas exchange and SPAD chlorophyll index measurements on the last day of the water deficit. To preserve leaf biochemical condition, collected leaves were immediately placed into liquid nitrogen, and then they were stored at 80°C. Each fresh frozen leaf was ground in liquid nitrogen on a pre-chilled mortar, and polyvinylpolypyrrolidone (PVPP) was added.

Enzymes were extracted from 0.2 g of macerated sample, which was homogenized in 1.5 mL of extraction buffer solution (100 mM potassium phosphate at 7.8, 0.1mM EDTA, and 10 mM ascorbic acid) and centrifuged at 13 000g for 10 min at 4°C, and the supernatant was collected (Biemelt et al., 1998). An enzyme extract aliquot was used to quantify the activity of superoxide dismutase (SOD) (Giannopolitis and Ries, 1977), catalase (CAT) (Havir and McHale, 1987), and ascorbate peroxidase (APX) (Nakano and Asada, 1981). Similarly, 0.3 g of macerated sample was used for hydrogen peroxide extraction. This sample was homogenized in 1.5 mL of 0.1% trichloroacetic acid (TCA) and centrifuged at 12 000g for 15 min at 4°C, and the supernatant was collected for quantifying hydrogen peroxide concentration (Velikova et al., 2000). Epoch^{TM} Microplate Spectrophotometer (Biotek, USA) was used to measure enzyme activities and H_2O_2 concentration.

After harvesting, the ground dry matter was obtained from the grains. For free amino acids and total proteins extraction, 0.20 g of the ground dry mass of grains was homogenized in 10 mL of potassium phosphate at pH 7.8 (extraction buffer solution), shaken with a VORTEX, placed at 40°C water bath for 30 min, and centrifuged at 3 360g (5 000 RPM) for 10 min, and the supernatant was collected to determine free amino acid (Yemm and Cocking, 1955) and total protein (Bradford, 1976) content by Epoch^{TM} Microplate Spectrophotometer (Biotek, USA).

2.5. Biometric measurements and chemical analysis

Quinoa was harvested at plant maturity when the grain moisture content reached 12 - 14% (99 DAT) (Gómez and Aguilar, 2016; Spehar and Santos, 2002). Shoots were harvested and

separated into leaves, stems, and grains. These plant organs were dried in a forced-air oven at 70°C for 48 h and their dry mas were weighed. The dry mass of grains and leaves were ground to be digested in HNO_3 following 3051A method (USEPA, 2007). Phosphorus, K, and Mg content were measured by ICP-OES and Se content was determined by atomic absorption spectrometry with graphite furnace atomization (GFAAS) (AAnalyst™ 800 AAS, Perkin Elmer). Nitrogen content was determined by digestion with sulfuric acid and titration by the Kjeldahl method (Malavolta et al., 1997). Two standard/certified reference materials - White Clover-BCR402 (Institute for Reference Materials and Measurements - IRMM, Geel, Belgium) and Peach Leaves-SRM 1547 (National Institute of Standards & Technology - NIST, Gaithersburg, MD, USA) – were added in each digestion batch to assure the QA/QC protocol for Se analyses, and Se recovery values were close to 91%.

2.6. *Statistical analysis and data processing*

Programming language R (R Core Team, 2022) was used for statistical analysis. Data were submitted to the analysis of variance (ANOVA), and their treatment means were compared by Tukey's test at 5% probability. In addition, the principal component analysis (PCA) of individuals was evaluated to study the combined effect of treatments on variables. The graphics were created, and regression models were fitted using the SigmaPlot for Windows software, version 11.0 (Systat Software Chicago, IL, USA).

3. Results

The interaction between soil water conditions and Se rates applied impacted significantly ($p \leq 0.05$) Se content in grains and leaves; Se accumulation and Se recovery by grains; stomatal conductance (g_s); transpiration rate (E); photosynthetic rate (A); instantaneous carboxylation efficiency (E_iC); water use efficiency (*WUE*); SPAD chlorophyll index; activity of superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX); hydrogen peroxide concentration; production of dry mass by grains and shoots; free amino acid and total proteins content in grains; and N, P, K, and Mg accumulation by grains.

3.1. *Selenium content, Se accumulation, and Se recovery percentage*

Increasing Se rates led to an increase of Se content in grains (Fig. 1a) and leaves (Fig. 1b), as well as Se accumulation (Fig. 1c) and Se-recovery (Fig. 1d) by grains. Different patterns were observed for these variables, allowing the predicting of maximum values of each one. Quadratic models described the response of Se content in grains and leaves and Se accumulation by grains in irrigated treatments, and linear models correlated them in water deficit treatments. Likewise, logarithmic models showed the relation between the percentage of Se-recovery by grains and Se rates in both soil water conditions, with initial increases - at low Se rate - followed by a stabilization (Fig. 1d).

Selenium contents in grains (Fig. 1a) were smaller than in the leaves (Fig. 1b). Also, fittings from linear models Se content in grains (Fig. 1a) and Se accumulation by grains (Fig. 1c) were close to those of their quadratic models. Contrarily, experimental points from Se content in leaves (Fig. 1b) at 2 mg kg^{-1} Se diverged, where the water deficit treatment was reduced by 47% when compared with its irrigated treatment.
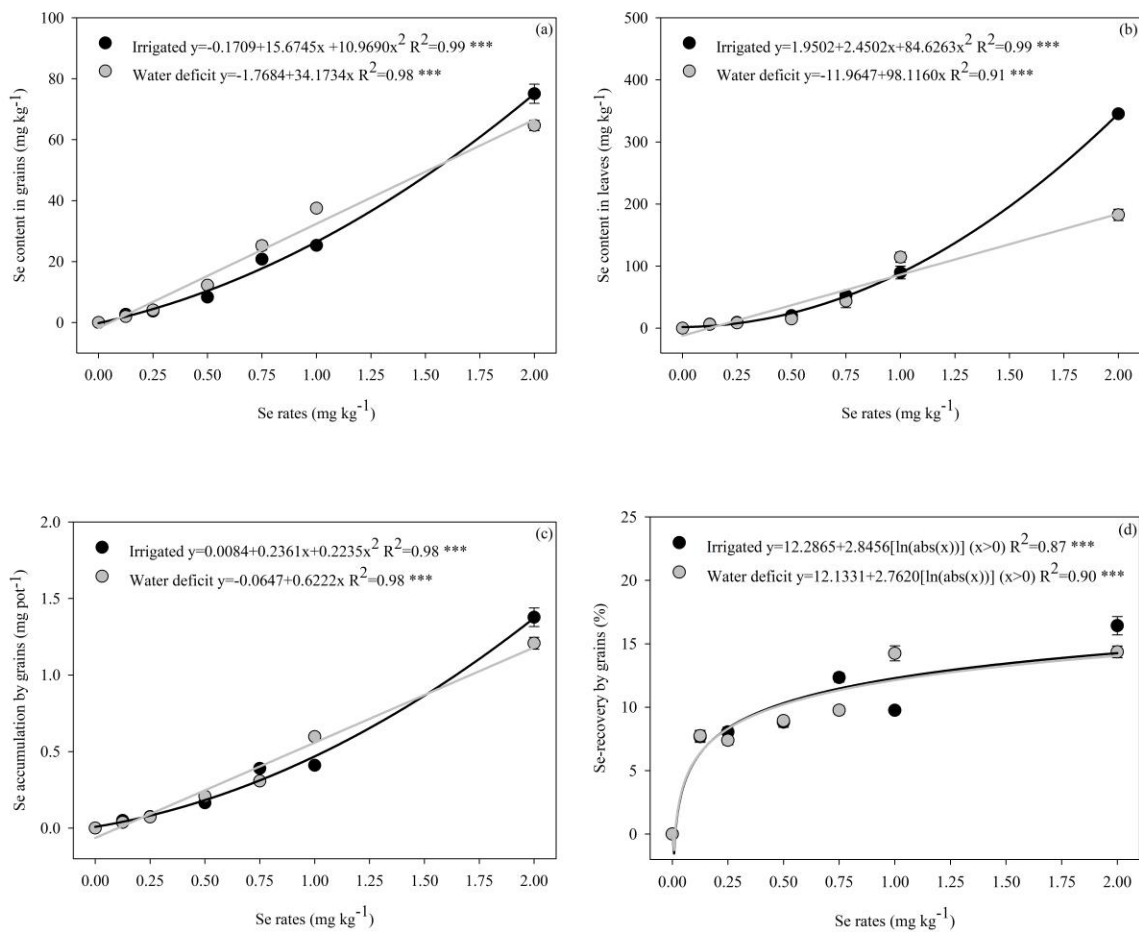

Fig. 1. Selenium content in grains (a) and leaves (b), Se accumulation (c), and Se recovery (d) by grains of quinoa in response to Se rates and soil water conditions. The vertical bars indicate standard errors of average values (n=4). Statistically significant differences are indicated: ***, $p \leq 0.001$.

3.2.   Gas exchange and SPAD chlorophyll index

Stomatal conductance (g_s) (Fig. 2a), transpiration rate (E) (Fig. 2b), photosynthetic rate (A) (Fig. 2c), and instantaneous carboxylation efficiency (E_iC) (Fig. 2d) diminished under water deficit; however, Se addition enhanced their activities, surpassing their irrigated treatments at Se rates where they reached their maximum activities. Stomatal conductance (Fig. 2a) and A (Fig. 2c) were highest at 0.75 mg kg^{-1} Se, followed by 1 mg kg^{-1} Se; still, the increase of g_s to 1 mg kg^{-1} Se was insufficient to differentiate it from its control. While E (Fig. 2b) and EiC (Fig. 2d) were only maximal at 0.75 mg kg^{-1} Se. On the other hand, in irrigated treatments, Se rates had no impact on g_s (Fig. 2a), whereas it kept E (Fig. 2b) and enhanced A (Fig. 2c) and E_iC (Fig. 2d) at 0.125 mg kg^{-1} and tended to keep their activities in other Se rates similar to their control.

Selenium did not influence water use efficiency (*WUE*) of irrigated plants (Fig. 2e). Conversely, Se improved *WUE* of water deficit plants at 0.25 mg kg^{-1} Se, followed by 0.5, 1, and 2 mg kg^{-1}, but the rise of *WUE* in the last three did not allow them to be differentiated from the control. Also, *WUE* at 0.25 and 1 mg kg^{-1} Se were higher than their irrigated treatments. SPAD chlorophyll index lessened in water deficit plants, but soil Se application at 0.5 mg kg^{-1} maximized the relative chlorophyll content of these stressed plants (Fig. 2f).
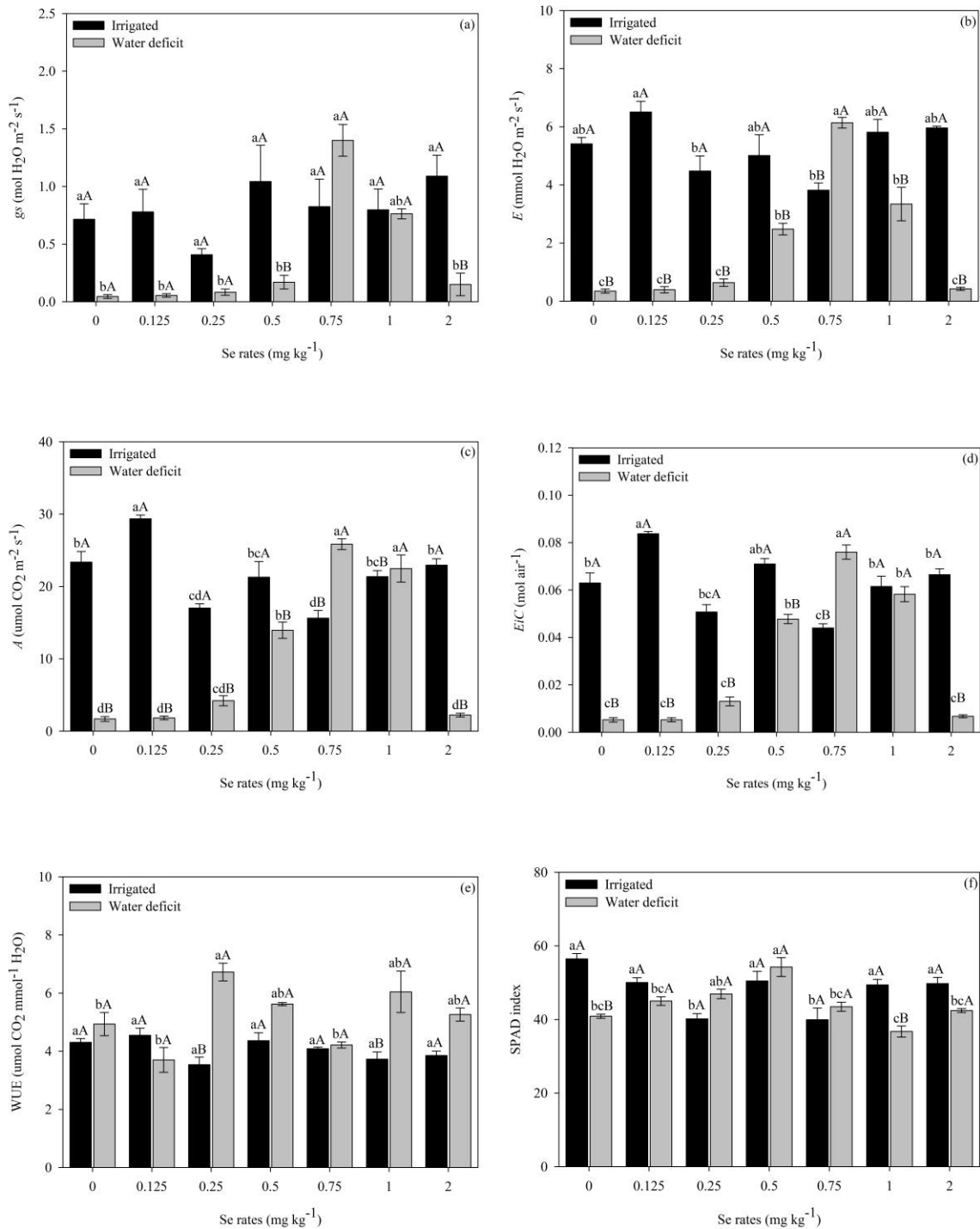
Fig. 2. Stomatal conductance - *gs* (a), transpiration rate - *E* (b), photosynthetic rate - *A* (c), instantaneous carboxylation efficiency - *EiC* (d), water use efficiency - *WUE* (e), and the SPAD index (f) in leaves of quinoa after water deficit. Uppercase letters compare the means of soil water conditions at each Se rate, and lowercase letters compare the means of Se rates at each soil water condition, according to Tukey's test (at 5% probability). Vertical bars indicate standard errors of average values: (a), (b), (c), (d), (e), and (f) n = 4.

3.3. *Antioxidant enzyme activity and hydrogen peroxide (H_2O_2) concentration*

Water deficit did not alter SOD (Fig. 3a) and APX activities (Fig. 3c); nevertheless, Se's presence did. SOD activity behavior did not show a definite trend among treatments, was maximal to 0.25 and 1 mg kg^{-1} Se, and was minimal to 0.125 mg kg^{-1} Se, also at 1 mg kg^{-1} Se was superior to its irrigated treatment. Meanwhile, APX activity increased in response to rising Se rates reaching its maximum activity to 2 mg kg^{-1} Se, followed by 0.5 and 1 mg kg^{-1} Se. Likewise, under water deficit, CAT activity (Fig. 3b) and hydrogen peroxide concentration (Fig. 3d) decreased by 39 and 42%, respectively, concerning their irrigated control treatments, but the presence of Se modified their behaviors. Selenium rates allowed CAT activity to equal those of their corresponding irrigated treatments and reach maximums to 0.25, 1, and 2 mg kg^{-1} Se, while hydrogen peroxide concentration was minimal to 0.75 mg kg^{-1} Se and maximal to 0.125 mg kg^{-1} Se.
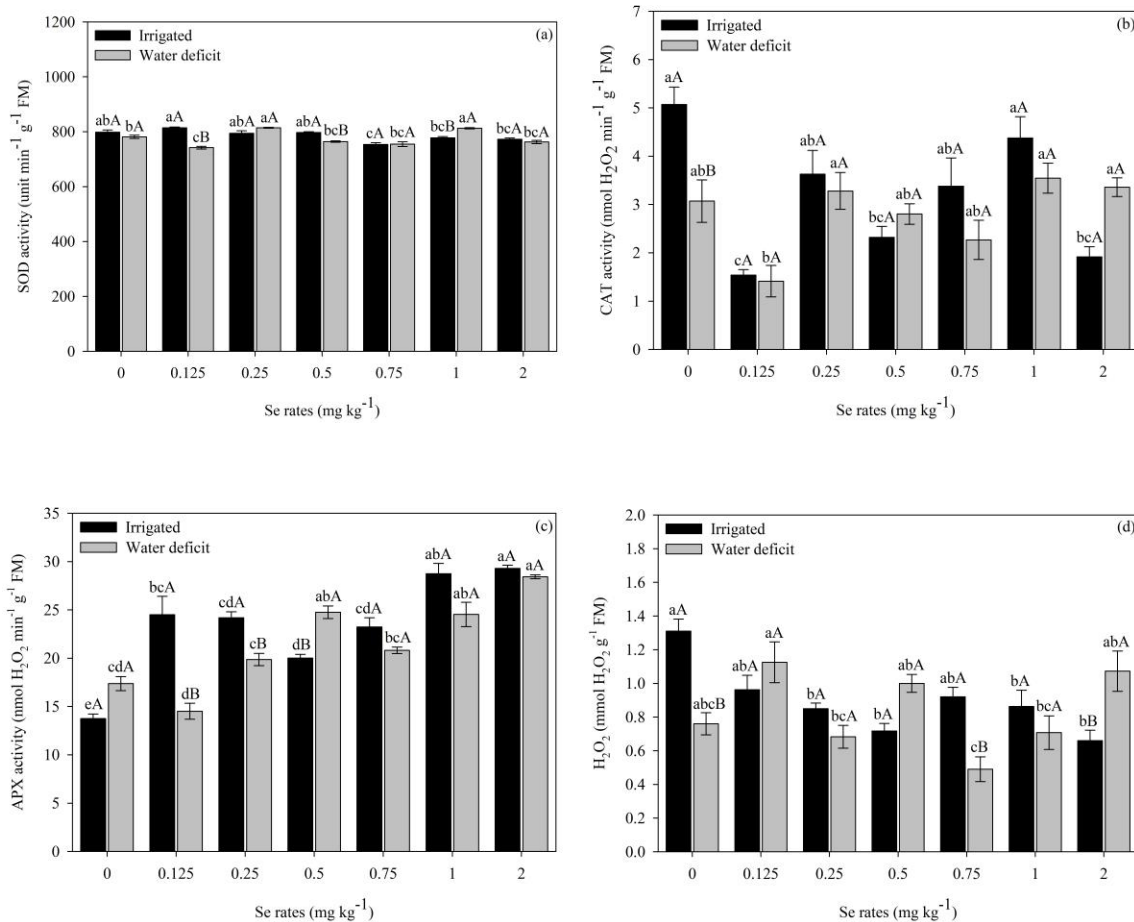
Fig. 3. Enzymatic activity of superoxide dismutase (SOD) (a), catalase (CAT) (b), and ascorbate peroxidase (APX) (c), and concentration of hydrogen peroxide (d) in the fresh mass (FM) of quinoa leaves after water deficit. Uppercase letters compare the means of soil water conditions at each Se rate, and lowercase letters compare the means of Se rates at each soil water condition, according to Tukey's test (at 5% probability). Vertical bars indicate standard errors of average values: (a), (b), (c), and (d) n = 4.

3.4.   Free amino acid and total protein contents in grains

Free amino acid (Fig. 4a) and total protein contents in grains (Fig. 4b) were not modified by water deficit; contrariwise, selenium influenced their accumulations. Under water deficit, the highest and lowest amounts of free amino acids accumulated at Se rates of 0.5 and 2 mg kg^{-1} (Fig. 4a), whereas total protein content did not change (Fig. 4b). In irrigated treatments, the Se rate at 0.25 mg kg^{-1} promoted the maximum accumulation of free amino acids, followed by 0.5 mg kg^{-1}; this last rise to 0.5 mg kg^{-1} did not differentiate it from its control (Fig. 4a). Meanwhile, a Se rate of 0.125 mg kg^{-1} led to the highest total protein content (Fig. 4b).
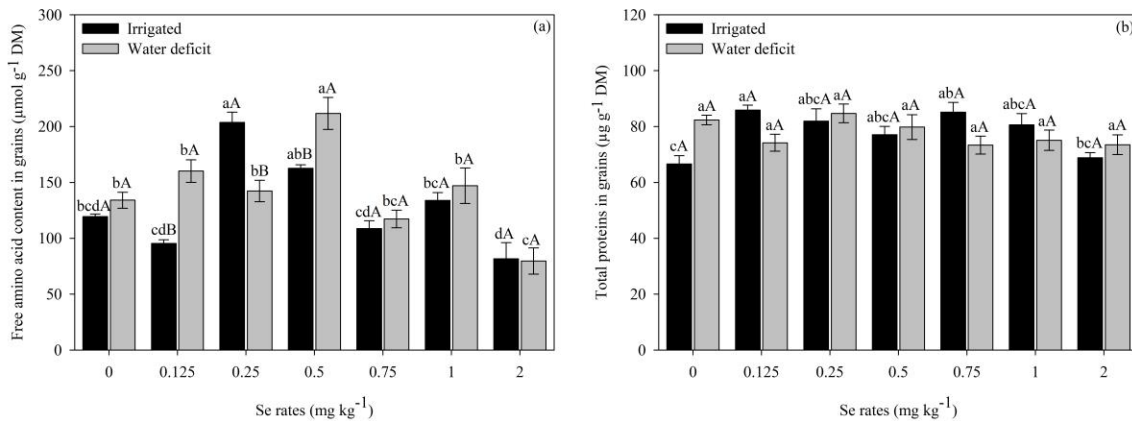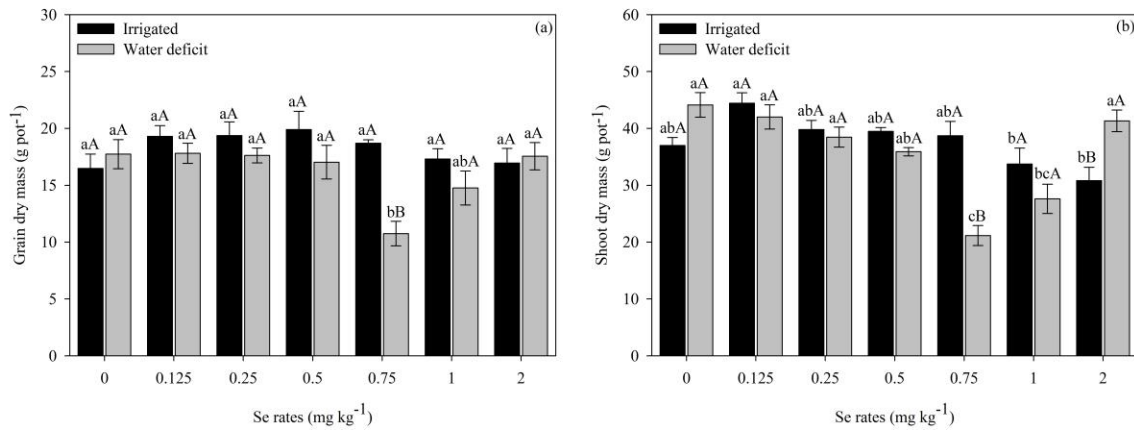

Fig. 4. Free amino acid (a) and total proteins (b) contents in dry mass (DM) of quinoa grains. Uppercase letters compare the means of soil water conditions at each Se rate, and lowercase letters compare the means of Se rates at each soil water condition, according to Tukey's test (at 5% probability). Vertical bars indicate standard errors of average values: (a) and (b) n = 4.

3.5.   Production of dry mass

Dry mass by quinoa grains (Fig. 5a) was similar between soil water conditions concerning the same Se rate, except for the rate of 0.75 mg Se kg^{-1}, where the water deficit treatment dropped 42% compared with its irrigated treatment. Selenium did not affect irrigated plants, whereas production of grains at water deficit reduced by 39% its dry mass at the 0.75 mg kg^{-1} Se rate compared with its control.

Water deficit did not influence shoot dry mass (Fig. 5b); nevertheless, the application of selenium did. Water deficit plants lost 52 and 37% of their shoot dry mass at 0.75 and 1 mg kg^{-1} compared with the control, whereas irrigated plants produced the highest shoot dry mass at 0.125 mg kg^{-1} Se and the lowest shoot dry mass at 1 and 2 mg kg^{-1} Se.

Fig. 5. Production of grain dry mass (a) and shoot dry mass (c) of quinoa. Uppercase letters compare the means of soil water conditions at each Se rate, and lowercase letters compare the means of Se rates at each soil water condition, according to Tukey's test (at 5% probability). Vertical bars indicate standard errors of average values: (a) and (b) n = 4.

3.6. Nutrient accumulation by grains

Quadratic models described N (Fig. 6a), P (Fig. 6b), and Mg accumulation by grains as a function of Se rates in both soil water conditions (Fig. 6d), where irrigated and water deficit treatment adjustments were close, and concave curves reached their minimum value at 1.2 mg kg^{-1} Se. On the other hand, K accumulation by grains in the two soil water conditions did not fit a model.

Accumulated amounts of N, P, K, and Mg by grains from water-deficient plants were similar to those of irrigated plants, except at 0.75 mg kg^{-1} Se, where their accumulation was reduced by 39, 37, 43, and 48%, respectively, compared with their irrigated treatments. Under water deficit, Se rates exceeding 0.5 mg kg^{-1} tended to decrease nutrient accumulation by grains.
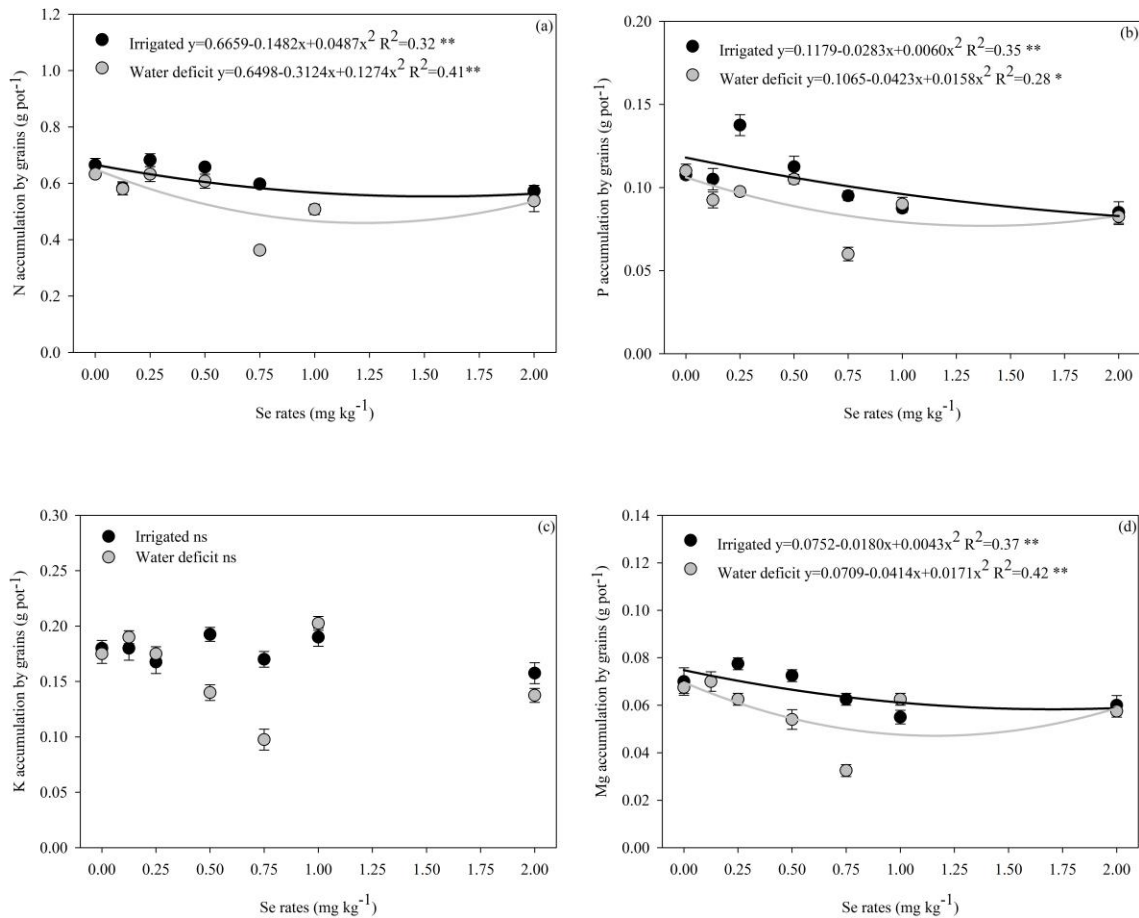
Fig. 6. Nitrogen (a), P (b), K (c), and Mg (d) accumulation by quinoa grains in response to Se rates and soil water conditions. Vertical bars indicate standard errors of average values (n = 4). Statistically significant differences are indicated: ns, *, **, ***, p≤0.001.

3.7. *Principal component analysis (PCA) of individuals*

Principal component analysis (PCA) evidenced that two principal components represented interaction among evaluated variables and explained 47.4% of the total variance (Fig. 7). The first (Dim1) and second (Dim2) components accounted for 31.0 and 16.4% variation, respectively. Shoot and grain dry mass, Se content in grains and leaves, Se accumulation and Se-recovery by grains, N, P, K, and Mg accumulation by grains, and APX activity contributed to (|cor|>0.5) Dim1; *A*, *E*, and E_iC correlated most strongly with (|cor|>0.5) Dim2, whereas g_s influenced significantly (|cor|>0.5) both components.

PCA for individuals differentiated groups by confidence regions at a 95% confidence level. The distance among ellipses allowed the identification of three different groups for each soil water conditions (I-0 to I-0.5, I-1, I-2, WD-0 to WD-0.5, WD-0.75, and WD-2). Likewise, the

interception of ellipses suggested similarity in the behavior among I-0.75, I-1, and WD-1; I-0.75, WD-0.5, and WD-1; and I-1, WD-0.75, and WD-1; and I-2 and WD-1.
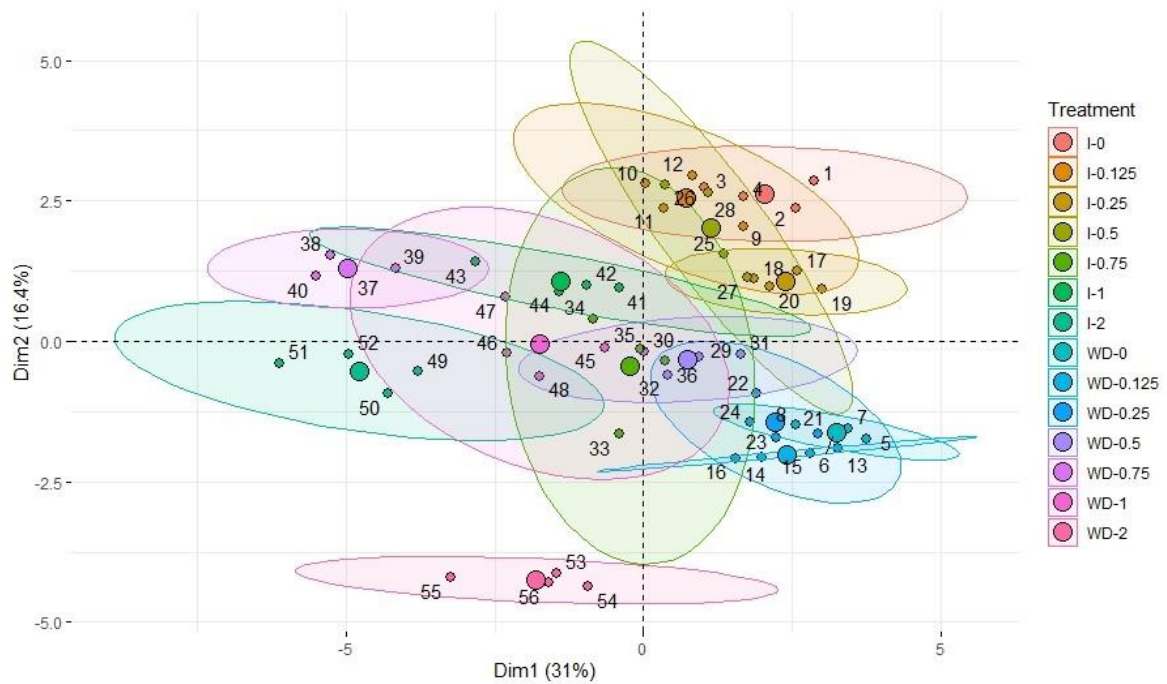
Fig. 7. PCA graph of individuals. Circles of the same color represent treatments of soil water conditions (irrigated (I) and water deficit (WD)) in response to Se rate (0.00, 0.125, 0.25, 0.50, 0.75, 1.00, and 2.00 mg kg^{-1}).

4. Discussions

Our study showed that the quinoa cultivar BRS Piabiru responded positively to soil Se application (Fig. 1). The Se content in quinoa grains increased (Fig. 1a), which improved the nutritional value of such grains, distinguishing them from the nutritional profile of grains reported in some field trials with quinoa varieties adapted to Argentina (Nascimento et al., 2014) and Ecuador (Ruales and Nair, 1993). Selenium increase in grains could be attributed to the combined effect of the chemical Se form and plant species. Selenate is highly soluble and bioavailable in agricultural soils (Hossain et al., 2021; Raina et al., 2021), including Oxisols (Schiavon et al., 2020), and is considered the best chemical form for increasing Se content in grains (Lanza and Reis, 2021). Besides, quinoa grains have Se accumulation capacity (Kitaguchi et al., 2008) since sulfate transporters enhance plant selenate uptake and transport (Hossain et al., 2021; Raina et al., 2021). Likewise, model estimates determined for Se content in grains (Fig. 1a) and leaves (Fig. 1b) and Se accumulation by grains (Fig. 1c) suggest that BRS Piabiru could be a quinoa cultivar tolerant to high Se rates. Indeed, biomass data from our study (Fig. 5) did not show detrimental effects of Se due to the possible pro-oxidative effect of

these high rates as reported by Khalofah et al. (2021). In addition, decreasing trends showed for Se-recovery by grains (Fig. 1d) for both soil water conditions suggested that quinoa self-regulated Se accumulation by its grains, which could be attributed to the lower availability of Se transporters due to the pro-oxidant Se action on the structure and function of these proteins (Raina et al., 2021) or their increased demand facing high Se rates.

Gas exchange evidenced that the water deficit negatively impacted quinoa photosynthetic physiology by reducing their stomatal conductance (g_s) (Fig. 2a), transpiration rate (E) (Fig. 2b), photosynthetic rate (A) (Fig. 2c), and instantaneous carboxylation efficiency (E_iC) (Fig. 2d) since they are a consequence of stomatal closure, one of the first responses to decreased water availability (Flexas et al., 2012). Nevertheless, quinoa growth plasticity and tissue elasticity give it inherent tolerance to water deficit (Hinojosa et al., 2018), which depends on the species, even on the genotype (Saddiq et al., 2021). Besides, the rapid stomatal closure is an isohydric behavior (Killi and Haworth, 2017) that could have been used by quinoa as one of the response mechanisms to maintain leaf water potential in its adaptation to face water deficit (Hinojosa et al., 2018) such as compatible solutes (da Silva et al., 2021). This mechanism possibly, promoted by ABA induced the decrease of turgor of stomata guard cells (Jacobsen et al., 2009). The decline in quinoa g_s (Fig. 2a) dropped E (Fig. 2b) and A (Fig. 2c), and this gas exchange behavior was consistent with the study of Jacobsen et al. (2009) and da Silva et al. (2021) findings for the cultivar BRS Piabiru at severe water deficit. The cultivar BRS Piabiru was adapted to tolerate water deficit and have high grain yield and biomass (Spehar et al., 2007) and is competent to genotypes currently developed tolerance to water deficit (da Silva et al., 2021). Consequently, in our study, the sensitivity of the cultivar BRS Piabiru to stomatal closure led lessen water loss and CO_2 assimilation reflected in lower photosynthetic activity but not in lower biomass production (Fig. 5) as reported in other studies in which physiological responses of the plant vary according to water deficit intensity and genotype (da Silva et al., 2021; Saddiq et al., 2021; Sun et al., 2014). Matmarurat et al. (2022) suggested that plants reserve food in tissues, which use them as backup energy under stress conditions, e.g., the high proline accumulation in drought-stressed plants might play other roles rather than the alleviation of osmotic stress.

According to the stages proposed by Flexas et al. (2012), water deficit triggered severe water stress in quinoa (g_s=45 mmol H_2O m^{-2} s^{-1}) (Fig. 2a). Selenium addition at the lowest rates (0.125 and 0.25 mg kg^{-1}) and from 0.5 to 2 mg kg^{-1} mitigated severe stress to moderate and mild levels, respectively, but since the studied factors did not influence the internal CO_2 concentration (C_i) (data non-shown), it could be assumed that g_s added to the action of

intermediates of the photosynthetic and respiratory pathways contributed to enhancing A (Fig. 2c). Therefore, g_s improvement exhibited the ability of low Se rates to stimulate quinoa's defense system, possibly through SOD, CAT, APX activities (Fig. 3a-c) and/or osmolyte accumulation (Fig. 4a) to enhance the gas exchange, which regulated its photosynthetic metabolism against less carbon fixation to ensure biomass production, as found by Khalofah et al. (2021). Likewise, quinoa's enzymatic antioxidant activity (Fig. 3a-c) in response to its oxidant activity (Fig. 3d) influenced g_s at high Se rates (1 and 2 mg kg^{-1}). Such behavior did not reflect toxicity, on the contrary, this effect led to turn into severe stress of quinoa to mild in different intensities, i.e., its g_s at 1 mg kg^{-1} was significantly enhanced close to the maximum value recorded, whereas its g_s at 2 mg kg^{-1} was increased just to reach the lower limit of mild stress. This difference in g$_s$ responses could elucidate that the slight variation at 2 mg kg^{-1} Se was insufficient to reach the photosynthetic adjustment capacity recorded at 1 mg kg^{-1} Se (Fig. 2b-d). Also, the best gas exchange performance at 0.75 mg kg^{-1} Se followed by 1 mg kg^{-1} Se (Fig. 2a-c) could be attributed to the integrated action intensity of the antioxidant enzyme system to mitigate oxidative stress (Fig. 3), which was evidenced in their higher E_iC (Fig. 2d).

On the other hand, similar behaviors of WUE in both soil water conditions without Se application (Fig. 2e) responded to the cultivar BRS Piabiru's ability to tolerate water deficit that enhances its WUE (Saddiq et al., 2021) through the proportional regulation of the relationship between A and E, which positively influences its biomass production (Fig. 5) despite the lower water availability, as was proved by da Silva et al. (2021). Besides, Se addition at low rates enhanced the water deficit tolerance of the cultivar BRS Piabiru by increasing its sensitivity to stomatal closure, e.g., with free amino acid accumulation (Fig. 4a), which led to a rise in its WUE by regulating its leaf water potential with osmolytes, as indicated by Khalofah et al. (2021). At the same time, WUE improvements at higher Se rates could also be explained by the response in the influence of antioxidant enzymatic and oxidant activities that affected g_s since high Se rates did not impair WUE and its rising at 1 mg kg^{-1} Se was more intensive than 2 mg kg^{-1} Se.

Water deficit restricted leaf gas exchange of the cultivar BRS Piabiru (Fig. 1a-c), reducing CO_2 assimilation, which disturbed the electron transport chain and diminished the efficiency of photosystem II (da Silva et al., 2021). Therefore, the drop in relative chlorophyll content in leaves at water deficit (Fig. 1f), also registered by Saddiq et al. (2021), could be related to the increase in oxidative stress triggered by electron transport unbalance, as this destabilizes thylakoid membranes decreasing chlorophyll biosynthesis and nutrient uptake (Hussain et al., 2019). On the other hand, the low Se rate at 0.5 mg kg^{-1} reversed the negative impact of water

deficit by promoting greater enzymatic (SOD, CAT, APX) (Fig. 3a-c) and non-enzymatic (free amino acids) antioxidant activity (Fig. 4a), which protected thylakoid and chlorophyll (Khalofah et al., 2021) showing increased SPAD index in quinoa leaves (Fig. 1f) and allowing continuity of nutrient accumulation by quinoa grains such as N (Fig. 6a) and Mg (Fig. 6d).

Selenium rates of 0.25 and 1 mg kg^{-1} significantly promoted SOD activity (Fig. 3a) in quinoa, counteracting the oxidative stress caused by water deficit through the superoxide ion dismutation that formed H_2O_2, a substrate that in turn enhanced the activities of their CAT (Fig. 3b) and APX (Fig. 3c) enzymes to scavenge ROS, which was validated by the decrease in H_2O_2 concentrations in the referenced rates (Fig. 3d); these antioxidant mechanisms are in agreement with those reported in the review by Lanza and Reis (2021). On the other hand, the exhibited CAT behavior (Fig. 3b) and the increasing APX activity (Fig. 3c) evidenced that Se not only induced their activities in quinoa to scavenge hydrogen peroxide formed by the SOD action. It is known that the main sources of H_2O_2 production are the electron transport chain in the chloroplast, mitochondria, endoplasmic reticulum, cell membrane, β-oxidation of fatty acid, and photorespiration, and also the water deficit favors photorespiration by reducing the CO_2 to O_2 ratio with stomatal closure resulting in the increased release of H_2O_2 through the oxidation of its glycolates in the peroxisome, where CAT is mostly active (Das and Roychoudhury, 2014). Moreover, Se increases the accumulation of ascorbate, a component of the non-enzymatic process that acts as a reducing agent in the APX reaction to detoxify hydrogen peroxide (Lanza and Reis, 2021).

Selenium application at 0.5 mg kg^{-1} in water deficit maximized free amino acid content in quinoa grains (Fig. 4a), allowing to manifest the activation of the non-enzymatic antioxidant defense system, which contributed to the joint action of antioxidant enzymes (Fig. 3a-c) in H_2O_2 scavenging (Fig. 3d) and the osmotic adjustment regulation by enhancing WUE (Fig. 2e). Additionally, it protected proteins from osmotic perturbations; thus, APX enzyme activity of quinoa increased enhancing its antioxidant action (Fig. 3c), and the thylakoid membrane preserved its integrity ensuring chlorophyll biosynthesis (Fig. 2f) and the photosynthetic process continuity (Fig. 2c), as pointed out by Khalofah et al. (2021). Total proteins at low water availability did not alter their quinoa grain contents in response to Se addition; on the contrary, they were similar to those of their irrigated treatments (Fig. 4b), which suggested that the cultivar BRS Piabiru uses amino acid and protein accumulations as inherent mechanisms of tolerance to water deficit (Saddiq et al., 2021) to achieve higher antioxidant activity to stabilize thylakoid membrane (Killi and Haworth, 2017).

Recorded hydrogen peroxide levels suggested that its moderate reactivity (Das and Roychoudhury, 2014) influenced more than one quinoa tolerance mechanism to water deficit involved directly or indirectly in its scavenging that did not include proline accumulation (data non-shown), where Se regulated the antioxidant activity, as observed by Khalofah et al. (2021). SOD, CAT, and APX enzymatic activities and the free amino acid presence were conjugated with the cultivar BRS Piabiru's ability to tolerate water deficit altering to different degrees the H_2O_2 concentrations. Thus, hydrogen peroxide level dropped drastically at 0.75 mg kg^{-1} Se, leading to the most favorable photosynthetic adjustment described above, and rose to a maximum when the antioxidant activity was insufficient to overcome the oxidative stress, perhaps increased by the pro-oxidant Se action.

In our study, the cultivar BRS Piabiru's ability to tolerate water deficit allowed grain production under this severe oxidative stress the same as in irrigated plants (Fig. 5a), showing a behavior opposite to that reported by other researchers for this cultivar (da Silva et al., 2021; Rêgo et al., 2017). Besides, its action was combined with the benefits of Se addition, increasing the production of energy that was manifested in the gas exchange potentiated by activating the antioxidant defense systems explained above. Nevertheless, the drastic lessening in grain dry mass to 0.75 mg kg^{-1} Se suggested that the energy produced at this rate was insufficient to sustain the growth and development processes of quinoa that maintained grain production as in its irrigation treatment and activated antioxidant mechanisms to cope with the severe oxidative stress, given that its enzymatic and non-enzymatic defense systems were prioritized as observed. At the same time, grain production at 1 mg kg^{-1} reflected a slight decline by deviation of this energy but did not differ from other Se rates. da Silva et al. (2021) found genotypes that reported higher photosynthesis without high yields, i.e., a higher photosynthetic capacity is not necessarily reflected in higher grain production because other factors are involved in grain filling, such as plant morphology and its ability to use photoassimilates. Furthermore, it was reported that water and energy conservation mechanisms to cope with water deficit are influenced by the plant species; for example, some plants conserve water to increase photosynthetic efficiency and maintain yields (Matmarurat et al., 2022). The accumulation behavior of shoot dry mass was similar to grain dry mass, but at 1 mg kg^{-1} Se, it diminished more accentuated (Fig. 5b), which could be attributed to low dry mass production in other organs.

Nitrogen (Fig. 6a), P (Fig. 6b), K (Fig. 6c), and Mg (Fig. 6d) accumulations by grains followed the same behavioral pattern of grain dry mass production since its quantification is based on this biomass. Water deficit did not negatively impact N, P, K, and Mg accumulation.

As found, the tolerance of this quinoa cultivar mitigates water restriction through the conjugation of enzymatic and non-enzymatic mechanisms identified in this study. This mechanism regulates the water potential of cells and enhances the photosynthetic efficiency by interacting with the defense activities promoted by low Se rates. These processes maintain the dynamics of cellular activity, thus absorbing and accumulating essential nutrients and Se in the plant (Zhou et al., 2020), except when the energy produced does not satisfy the requirements for grain production and plant protection.

Groups delimited by the ellipses (Fig. 7) elucidated the understanding of factor effects under study. Responses of the variables in WD-1 were highly unpredictable and tended to behave as I-0.75, I-1, and I-2. I-2 is the highest Se rate, and some variables, except enzymes and H_2O_2, responded to its application similarly to their control treatment, i.e., it was possibly detrimental to variables due to its pro-oxidative effect, or there was no effect. On the contrary, variables in WD-0.75 behaved like their irrigated treatment at 1 mg kg^{-1} Se. These behaviors suggested that the best Se rate to mitigate oxidative stress caused by water deficit is 0.75 mg kg^{-1}.

5. Conclusions

Our findings showed that soil Se biofortification enhanced the nutritional quality of quinoa grains with selenium. Also, Se potentiated the antioxidant defense system of quinoa cultivar BRS Piabiru by increasing the enzymatic activity of SOD, CAT, and APX and free amino acid accumulation to cope with water deficit. The coordinated expression of those tolerance mechanisms would mitigate severe oxidative stress resulting in gas exchange alterations that regulate the photosynthetic metabolism. Besides, at 0.75 mg kg^{-1} Se, the effect of these antioxidant mechanisms maximized stomatal conductance (g_s), transpiration rate (E), photosynthetic rate (A), and instantaneous carboxylation efficiency (E_iC) and minimized the hydrogen peroxide concentration.

CRediT authorship contribution statement

Ruby Vega-Ravello: Conceptualization, methodology, validation, formal analysis, investigation, data curation, writing – original draft, writing – review & editing. **Cynthia de Oliveira:** Supervision, writing – review & editing. **Luiz Roberto Guimarães Guilherme:** Resources, supervision, writing – review & editing. **Guilherme Lopes:** The project advisor, planned and supervised all phases of this research. Funding acquisition.

Declaration

Conflicts of interest The authors declare that they have no conflicts of interest.

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FINAL CONSIDERATIONS

We know that agriculture ensures world food security; however, we are unaware of it because we have not a proper understanding of agricultural issues and how they impact our lives. Agriculture is the most important human activity, especially during the last pandemic event of COVID-19 and current international conflicts. Although the agricultural production is constantly threatened by nature or human activities; agriculture supplies food to nearly 8 billion people worldwide. In this context, our efforts should focus on the developing and driving a sustainable crop production. Also, agricultural technologies should adapt to diverse production conditions, such as agronomic biofortification. Thus, we would contribute to food security.

Our three studies reached increasing the Se content in grains, confirming that biofortification is the way for nutrient enhancement of staple foods. Thus biofortification may be an effective agricultural strategy to biofortify grains on other important crops. Also, we found that low Se rates enhance plant tolerance mechanisms against water deficit. The enzymatic antioxidant activity increased to cope with water deficit, where SOD exhibited its action as the first defense barrier to mitigate oxidative stress generated by reactive oxygen species and provide the substrate that promotes CAT and APX activities. At the same time, non-enzymatic antioxidant activity was represented by compatible solute accumulation, such as proline and free amino acids, suggesting that they acted like osmolytes to regulate leaf water potential and osmoprotectants to prevent protein degradation. The coordinated action of selenium's beneficial mechanisms was observed through improving physiological and biochemical processes such as gas exchange (stomatal conductance, transpiration rate, and CO_2 assimilation rate) and instantaneous carboxylation efficiency, which suggested that antioxidant systems protected chloroplasts and helped regulate the continuity of photosynthetic process to achieve biomass production (Fig. 1). Conversely, high rates of Se acted as pro-oxidants, increasing oxidative stress manifested in the increase of the hydrogen peroxide concentration.
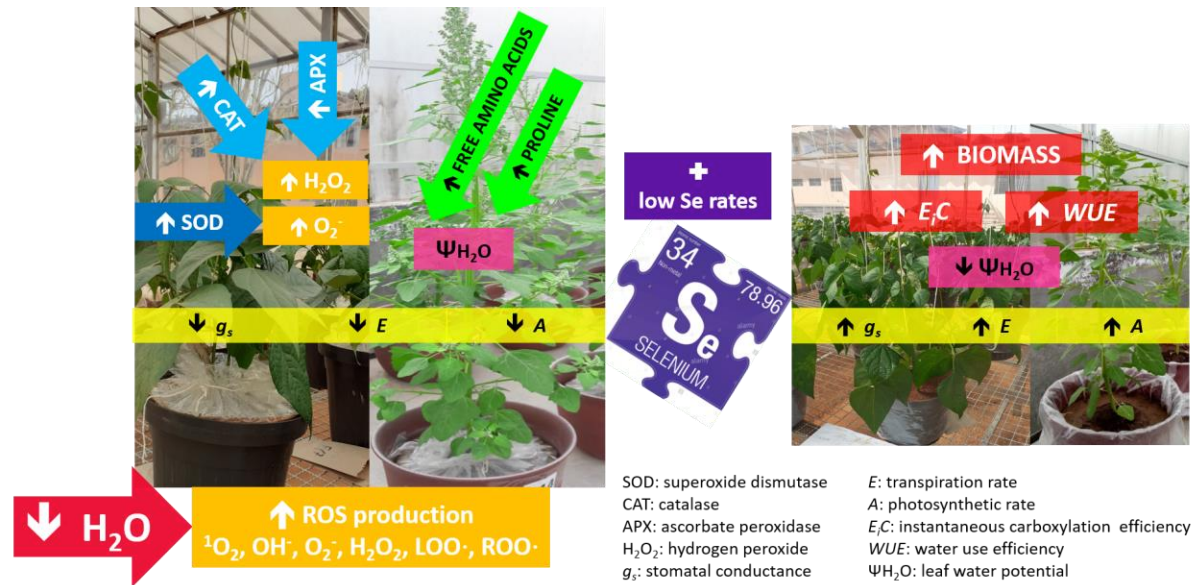
Fig. 1. Physiological and biochemical response to non-enzymatic and enzymatic antioxidant activities

Low Se rates used for agronomic biofortification of common bean and quinoa through the soil had a positive impact on crops. Plants' behavior evidenced that Se biofortification had a dual purpose, on the one hand, to increase the nutritional value of grains for the human diet and, on the other hand, to enhance plant tolerance to mitigate the water deficit effects, which is a promising benefit despite these Se rates applied through the soil requires more Se than that used in efficient foliar biofortification of Se.

This doctoral thesis has provided basic information on the capacity of selenium accumulation by common bean and quinoa grains and the potential for tolerance to water deficit that these crops can develop. Nevertheless, the next challenge will be to set up field experiments to investigate if other factors modify the behavior trends obtained in greenhouse results. Likewise, more studies should carry out to adjust results because they cannot extrapolate to field conditions. These new studies could consider foliar fertilization to determine the Se rate that has the best response to biofortification and tolerance to water deficit, thus, adding another advantage to this efficient Se application. Besides, since there are different physiological and biochemical mechanisms, should include biochemical analyses of other enzymes and solute compatibles to know if other antioxidant pathways work in coordination with mechanisms found. At the same time, more readings of gas exchange should be made before, during, and after the water deficit to establish its behavior during this critical period.