PATRICIANI ESTELA CIPRIANO

SELENIUM BIOFORTIFICATION STRATEGIES AND THEIR EFFECT ON PLANT NUTRITION AND ANTIOXIDANT METABOLISM IN SORGHUM GENOTYPES CULTIVATED IN TROPICAL SOILS

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ESTRATÉGIAS PARA A BIOFORTIFICAÇÃO COM SELÊNIO E SEU EFEITO NO NUTRIÇÃO DE PLANTAS E METABOLISMO ANTIOXIDANTE EM GENÓTIPOS DE SORGO CULTIVADO EM SOLOS TROPICAIS

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À minha mãe Heloisa,
Ao meu pai Aguinaldo,
À minha irmã Hellen,
À minha avó Maria Julia (in memoriam),
Ao meu avô Othoniel (in memoriam),
Dedico.

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RESUMO

O *Sorghum bicolor* L. é o quinto cereal mais importante do mundo sendo precedido por trigo, arroz, milho e cevada. O selênio (Se) pode apresentar propriedades essenciais ou tóxicas para animais e humanos dependendo da quantidade presente no alimento ingerido. Em todo o mundo, existem tanto áreas onde o Se no solo pode ser considerado deficiente quanto áreas onde esse elemento pode ser considerado como tóxico, sendo ampla a faixa de variação desse elemento nos ecossistemas. Como o sorgo é o alimento básico para milhões de pessoas no mundo, coincidindo com áreas onde ocorre deficiência de Se, a biofortificação com Se pode melhorar consideravelmente a nutrição de Se em humanos que vivem nessas áreas. Neste contexto, o presente trabalho teve como objetivos: i) avaliar a eficiência nutricional do Se aplicado ao sorgo via solo e via foliar; ii) comparar fontes e doses de Se para o sorgo em condições de campo e casa de vegetação, visando seu uso na alimentação humana e animal; e, iii) avaliar os efeitos do Se no metabolismo antioxidante, proteínas, carboidratos, teores de elementos minerais e rendimentos em plantas de sorgo. No primeiro estudo, foi feita uma aplicação de Se via solo, sendo a cultivar BM737 cultivada em casa de vegetação. O fornecimento de Se ocorreu por meio de diferentes doses de Se (0, 60, 240 e 480 µg dm^{-3}) e diferentes fontes de Se (SeA – potássio hidroxi seleneto, SeB – metil hidroxi seleneto, SeC – hidroxi seleneto, e SeD – selenato de sódio). No segundo estudo, foram feitas duas aplicações foliares de concentrações iguais de Se com cultivos em casa de vegetação e em campo (Lavras e Lambari) com oito cultivares (BM737, BRS310, Enforcer, K200, Nugrain320, Nugrain420, Nugrain430 e SHS410). No segundo estudo o Se foi fornecido em diferentes doses (0; 10, 20 e 40 g ha^{-1} de Se na forma de selenato de sódio) e no terceiro estudo o Se foi fornecido com diferentes fontes (controle – sem selênio; SeA – selenato de sódio, SeB – potássio hidroxi seleneto, SeC – acetil seleneto (ambos na dose de 10 g ha^{-1} de Se). Ambas as formas de aplicação do Se (via solo ou foliar) afetaram positivamente o metabolismo antioxidante e o teor mineral das cultivares. Houve também aumento no teor de Se nos grãos e na parte aérea das plantas de sorgo. Com a aplicação de Se via solo, a maior eficiência de absorção pelas raízes e recuperação do Se ocorreu com selenato. A aplicação de Se aumentou o conteúdo de carboidratos. A biofortificação de Se por meio de metil hidroxi seleneto ou hidroxi seleneto com 240 µg Se dm^{-3} e selenato de sódio com 60 µg Se dm^{-3} resultou em teores aceitáveis desse elemento no sorgo. O Se via foliar foi mais eficiente com selenato em baixas doses de Se. O sorgo responde positivamente à aplicação de Se.

Palavras-chave: *Sorghum bicolor* L. Biofortificação agronômica. Selenato. Seleneto. Compostos bioativos.

ABSTRACT

Sorghum bicolor L. is the fifth most important cereal in the world, being preceded by wheat, rice, maize, and barley. Selenium (Se) can have essential or toxic properties for animals and humans depending on the amount present in the ingested food. All over the world there are areas where soil Se can be considered deficient and other areas where it can be considered toxic, i.e., the range of soil-Se variation is very wide. As sorghum is the staple food for millions of people around the world, coinciding with areas where Se deficiency occurs, biofortification with Se can considerably improve Se nutrition in humans living in these areas. In this context, this study aimed to: i) evaluate the nutritional efficiency of Se provided to sorghum plants via soil and foliar application; ii) compare Se sources and doses to sorghum under field and greenhouse conditions, aiming its use in human and animal nutrition; and, iii) evaluate the effects of Se on antioxidant metabolism, proteins, carbohydrates, mineral elements and yields in sorghum plants. In the first study, an application of Se was made via soil, with cultivar BM737 being cultivated under greenhouse conditions. The supply of Se occurred through different doses of Se (0, 60, 240, and 480 µg dm^{-3}) and different sources of Se (SeA - potassium hydroxy selenide, SeB - methyl hydroxy selenide, SeC - hydroxy selenide, and SeD – sodium selenate). In the second study, two foliar applications of equal Se concentrations were carried out under greenhouse and field cultivation (Lavras and Lambari) with eight cultivars (BM737, BRS310, Enforcer, K200, Nugrain320, Nugrain420, Nugrain430 and SHS410). In the second study Se was provided at different doses (0, 10, 20, and 40 g ha^{-1} of Se in the form of sodium selenate) and in the third study Se was provided with different sources (control – no selenium; SeA – sodium selenate, SeB – potassium hydroxy selenide, SeC – acetyl selenide (both at a dose of 10 g ha^{-1} of Se). Both forms of Se application (via soil or foliar) positively affected the antioxidant metabolism and mineral content of the tested sorghum cultivars. It also increased the Se content in grains and shoots of sorghum plants. With the application of Se via the soil, the highest efficiency of absorption by the roots and recovery of Se occurred with selenate. The application of Se increased the carbohydrate content. Biofortification of Se using methyl hydroxy selenide or hydroxy selenide with 240 µg Se dm^{-3} and sodium selenate with 60 µg Se dm^{-3} resulted in acceptable levels of this element in sorghum plants. Selenium via foliar was more efficient with selenate in low doses of Se. Sorghum responds positively to the application of Se.

Keywords: *Sorghum bicolor* L. Agronomic biofortification. Selenate. Selenide.

Bioactive compounds.

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LISTA DE ABREVIATURAS

Se	Selênio
S	Enxofre
P	Fosforo
K	Potássio
Ca	Calcio
Mg	Magnésio
Fe	Ferro
Zn	Zinco
Cu	Cobre
Mn	Manganês

LISTA DE SIGLAS

FAOSTAT	Food and Agriculture Organization of the United Nations
USDA	United States Department of Agriculture
CONAB	Companhia Nacional de Abastecimento
OMS	Organização Mundial de Saúde

SUMÁRIO

PRIMEIRA PARTE

1 INTRODUÇÃO

O sorgo (*Sorghum bicolor* L.) é o quinto cereal mais cultivado no mundo, superado apenas por milho, trigo, arroz e cevada (FAOSTAT, 2020). Apresenta variação quanto ao seu uso em cada região do mundo, sendo utilizado na nutrição humana na África e na Ásia; entretanto, nas Américas e na Austrália, é usado como componente de ração animal e produção de etanol (PAIVA et al., 2017). Os cereais são comumente usados em programas de biofortificação por serem a base alimentar de dietas (TAYLOR; TAYLOR; KINI, 2012).

A introdução progressiva do sorgo na alimentação humana se deve principalmente à ausência de glúten em sua composição (PONTIERI et al., 2013), e também por ter efeito redutor do colesterol, ação anti-inflamatória, digestibilidade lenta e bloqueio da expansão de células cancerosas no esôfago e cólon humano (AWIKA et al., 2009). O sorgo pode ser consumido por meio de grão integral, farinha ou cereal matinal (ANUNCIAÇÃO et al., 2016; KHAN et al., 2015). Awika e Rooney (2004) afirmam que o grão de sorgo inteiro é uma fonte de carboidratos, fibras, compostos bioativos (ácidos fenólicos e antocianinas), amido e minerais. A biofortificação tem buscado elevar os níveis de minerais essenciais nos alimentos, por meio de intervenção agronômica e seleção genética (WHITE; BROADLEY, 2005).

O teor dos nutrientes varia de acordo com o estado nutricional das plantas como um todo. Os nutrientes podem ter relações sinérgicas e, ou, antagônicas com o Se, o que pode melhorar a nutrição das plantas e aliviar estresses abióticos, como: seca, altas temperaturas, salinidade e metais pesados (GUERRERO et al., 2014). Diferentes espécies de plantas têm respostas fisiológicas variáveis ao Se. Solos com baixas concentrações de Se causam baixa concentração deste elemento nas plantas, sendo assim, a deficiência de Se é observada nessas áreas, e consequentemente ocorre em pessoas em todo o mundo (SCHIAVON et al., 2016).

O Se é um micronutriente para a nutrição animal e humana (TERRY et al., 2000). A relevância desse elemento se dá por suas propriedades antioxidantes e anticancerígenas (THIRY et al., 2012). Devido ao papel do Se no processo antioxidante, o mesmo contribui para o funcionamento normal do sistema imunológico e tireoidiano (FAIRWEATHER-TAIT et al., 2011). Os oligoelementos essenciais inter-relacionam-se com proteínas na forma de cofatores. No entanto, o Se atua diferentemente, pois é co-translacionalmente incorporado na cadeia polipeptídica como parte do 21º aminoácido de ocorrência natural, selenocisteína, codificado pelo códon UGA (ZOIDIS et al., 2018). Pequenas quantidades de Se são necessárias para manter uma boa saúde, mas a ingestão excessiva desse nutriente pode causar

problemas de saúde (COZZOLINO, 2000).

Em relação às plantas, sua essencialidade ainda não foi confirmada. No entanto, os efeitos benéficos ocorrem em concentrações adequadas. Exemplos de efeitos benéficos do Se em plantas são: aumento da produtividade em arroz (BOLDRIN et al., 2013); maior atividade de enzimas do sistema antioxidantes em arroz (FENG et al., 2016), e atrasos em alguns dos efeitos da senescência, além de poder melhorar a utilização da luz em comprimentos de onda curto em alface (HARTIKAINEN, 2005). Porém, o limite de toxicidade, o impacto sobre o teor de minerais e a forma química do Se devem ser considerados para evitar um declínio no rendimento da cultura (LONGCHAMP et al., 2015; POBLACIONES et al., 2014).

A biofortificação com Se pode ser feita por meio do fornecimento de Se via foliar e, ou, via solo. A biofortificação do Se por meio de fertilizantes foliares tem sido praticada em países como Finlândia, Reino Unido, Malawi e China, que obtiveram aumento no teor de Se dos grãos cultivados pela aplicação foliar de selenito ou selenato (CHILIMBA et al., 2012; EUROLA et al., 1991; FANG et al., 2008; RAYMA, 2008). A fertilização foliar é mais eficiente do que via solo porque a absorção de Se é mais eficaz, pois não há interferência da matriz do solo (KOPSELL et al., 2009).

Em solos tropicais, Lessa et al. (2020) observaram que com a aplicação foliar de Se em arroz quando comparado ao fornecimento de Se via solo foi necessária uma dose de Se menor para atingir o consumo de Se recomendado. Demonstrando assim a maior eficiência da aplicação foliar em relação à aplicação via solo nesse ambiente de cultivo. Porém, são escassos os estudos com a aplicação de Se orgânico, principalmente com aplicação foliar. Wang et al. (2019) observaram que o Se pode ser transportado por plantas de milho com a aplicação de Se orgânico, especialmente por metil-selenocisteína.

O sucesso da biofortificação para enriquecimento de plantas com Se depende de vários fatores como: fonte de Se, dose de Se, o modo de fertilização, espécies de planta, estádio fisiológico de aplicação de Se, entre outros. Neste contexto, o presente trabalho tem como objetivos: i) avaliar a eficiência da Se aplicado via solo e via foliar para a biofortificação do sorgo; ii) comparar fontes e doses de Se em condições de campo e casa de vegetação visando atingir quantidades de Se adequadas para a alimentação humana e animal; e, iii) avaliar os efeitos do Se no metabolismo antioxidante, proteínas, carboidratos, teor de nutrientes e rendimentos em plantas de sorgo.

2 REFERENCIAL TEÓRICO

2.1 Características Gerais do Sorgo

O sorgo é uma planta da família Poaceae, gênero *Sorghum* e espécie *Sorghum bicolor* L. Moench. O sorgo é o quinto cereal mais cultivado no mundo, superado apenas por milho, trigo, arroz e cevada (FAOSTAT, 2020). Estima-se que 500 milhões de pessoas no continente africano e asiático consumam o sorgo como alimento básico (MUTISYA et al., 2009). Os principais países importadores são China, México, Japão e Chile. A China tem se destacado na importação por usar o sorgo como alternativa na produção de ração animal (BOTELHO; RAMOS; ISAAK, 2018).

A produção mundial de sorgo na safra 2019/20 ficou em torno de 58,283 milhões de toneladas. Entre os principais produtores mundiais estão os EUA com 8,673 milhões de toneladas, seguido de Nigéria e Etiópia com 6,665 e 5,266 milhões de toneladas, respectivamente. O Brasil teve a nona maior produção na safra 2019/20 com 2,498 milhões de toneladas (USDA, 2021). Para a safra 2020/21 estima-se a produção de 2,3 milhões de toneladas em 864,5 mil hectares (CONAB, 2021).

O sorgo cultivado no Brasil em grãos é destinado à indústria de ração animal, e, quando forragem, é destinado à alimentação dos ruminantes (ALBUQUERQUE, et al. 2019). Originário da África, foi introduzido no Brasil no século XX e apresentava como principais estados produtores Goiás e Minas Gerais. Atualmente, os principais estados produtores são Goiás, Minas Gerais, Pará, Tocantins, Piauí, Rio Grande do Norte, Paraíba, Bahia, Mato Grosso, Mato Grosso do Sul, Distrito Federal e São Paulo (CONAB,2021).

No Centro-Oeste brasileiro, é comum ocorrerem períodos com baixos índices pluviométricos e altas temperaturas, o que agrava as condições adversas para a segunda safra. Essas características, muitas vezes, tornam arriscado o cultivo com milho em segunda safra, notadamente quando em plantios tardios, ocasião em que se sai da "janela" comum de plantio desta gramínea. Esse cenário proporciona ao sorgo condições para sobressair em seu potencial em relação ao milho (SILVA et al., 2015). Devido a oscilação no volume e frequência das chuvas, é recomendado a semeadura logo após a colheita da soja nas regiões de Cerrado. no início da estação chuvosa nas regiões do semiárido (ALBUQUERQUE, et al. 2019).

O sorgo é uma espécie tropical de dias curtos exigente em clima quente, cuja temperatura ideal para seu desenvolvimento oscila entre 33 a 34ºC, sendo sua produtividade afetada negativamente em temperaturas acima de 38ºC e abaixo de 16ºC (BARBOSA, 2007) e

necessita de 380 a 600 mm de água durante o ciclo (SANS et al., 2003). Enquanto que o milho necessita de 25°C a 30°C de temperatura diurna ótima, sendo que quando no estádio de 12-14 folhas, temperaturas acima de 32°C e abaixo de 19°C reduzem a produção, e necessita de 500-800 mm de água durante seu ciclo (MIRANDA et al., 2019). Além disso, em comparação com o milho e a *Brachiaria decumbens*, o sorgo apresenta a maior taxa fotossintética e balanço de biomassa favorável, sendo a espécie que apresenta o melhor desempenho ecofisiológico sob limitação hídrica (SANTOS et al., 2014).

O sorgo destaca-se dentre os sistemas agrícolas implantados no Brasil por tolerar condições desfavoráveis de umidade do solo e produzir grande quantidade de matéria seca com relação carbono/nitrogênio relativamente elevada (DAN et al., 2010). Os mecanismos fisiológicos que a planta de sorgo possui faz com que seja mais tolerante ao estresse hídrico o que permite adaptação a ambientes secos e quentes, os quais são limitantes para o cultivo de outras espécies (BUSO et al., 2011). Dentre os mecanismos relacionados à resistência e à seca, o sorgo apresenta características de tolerância e escape. A tolerância está relacionada com a diminuição do metabolismo e o escape está relacionado ao sistema radicular profundo e ramificado o que permite melhor eficiência na extração de água do solo (MAGALHÃES et al. 2014).

É uma planta autógama, com baixa taxa de fecundação cruzada, apresenta metabolismo C4 com altas taxas fotossintéticas. A inflorescência é do tipo panícula, com eixo central ou ráquis, que possui diversas formas, de acordo com as diferentes variedades. Devido às suas características xerofíticas e ao eficiente mecanismo morfológico, a planta de sorgo tem habilidade de se manter dormente durante o período de seca, restabelecendo seu crescimento imediato após condições favoráveis (VON PINHO; FIORINI; SANTOS, 2014).

O sorgo é classificado quanto às características agronômicas em 5 tipos: forrageiro, utilizado na produção de silagem; sacarino para produção de açúcar e etanol; granelífero para produção de grão, adaptado à colheita mecânica; corte e pastejo usado para pastejo extensivo e o tipo usado para produção de vassouras. A diferença entre eles está na altura e proporção de colmo, folhas e panículas, o que reflete na produtividade, na composição bromatológica e no valor nutritivo da planta (VON PINHO; FIORINI; SANTOS, 2014).

A cultura do sorgo possui características semelhantes à do milho na sua composição química e no seu valor nutritivo, porém, apresenta vantagens quanto à exigência em fertilidade do solo, resistência à estiagem, tolerância a pragas e doenças, e perfilhamento obtendo boa capacidade de rebrota (PINTO, 2008). Dentre as cultivares de sorgo disponíveis no mercado, o uso de híbridos simples tem predominado. Esses materiais apresentam ampla

adaptabilidade e estabilidade de produção. Características como altura da planta, diâmetro do colmo e ângulo foliar são utilizadas no intuito de selecionar cultivares adaptados a cada região, assegurando boa produtividade (TARDIN et al., 2012). Os híbridos têm diferenças quanto às características agronômicas e valor nutritivo que fazem com que haja variação na produtividade. Além da escolha do híbrido, fatores como local de cultivo (ALBUQUERQUE et al., 2019) e adubação (SANTOS et al., 2014) podem afetar a produtividade da planta.

Segundo Von Pinho, Fiorini e Santos (2014), o ciclo do sorgo pode ser dividido em três fases: vegetativa, reprodutiva e período de maturação dos grãos. A etapa de crescimento EC1 é caracterizada pela germinação, aparecimento da plântula, crescimento das folhas e estabelecimento do sistema radicular fasciculado. A fase seguinte EC2 inicia-se quando o meristema apical se diferencia em um meristema floral, e continua com o desenvolvimento da inflorescência até a antese. Nessa fase, há uma elongação rápida dos entrenós do colmo e grande expansão das folhas. A terceira etapa EC3 vai da floração à maturação fisiológica e senescência de parte das folhas.

Durante os primeiros 20 ou 30 dias após a emergência, as plantas crescem lentamente; depois, inicia-se o período de crescimento sendo compreendido entre 30 a 59 dias após a embebição da semente. Entre 30 e 40 dias da emergência inicia-se a fase de diferenciação floral, onde a planta deixa de produzir as partes vegetativas para formar partes reprodutivas, nesta fase o acúmulo de matéria seca é rápido. Após esse período, inicia-se a fase de emborrachamento, onde há um rápido alongamento do colmo e da panícula, que se completa em torno dos 50 a 55 dias aproximadamente. Após a emissão da panícula, entre 60 a 70 dias após a emergência da planta, se dá o florescimento e aproximadamente aos 90 dias após a germinação, atinge a maturidade fisiológica (VON PINHO; FIORINI; SANTOS, 2014).

A maturidade fisiológica da planta de sorgo é caracterizada por unidade de 30 a 35% (bulbo úmido - b.u.) nos grãos, presença de uma camada preta (“black layer”) entre o endosperma basal e a área vascular do pedicelo dos grãos, e plantas totalmente secas (FORNASIERI FILHO; FORNASIERI, 2009). A maturidade fisiológica também pode ser definida como o máximo de matéria seca acumulado pelas sementes, ou seja, pelos grãos (FORNASIERI FILHO; FORNASIERI, 2009).

Sendo assim, o conhecimento do padrão diferencial de acúmulo de matéria seca e de nutrientes, durante o ciclo da cultura do sorgo, permite avaliar as necessidades de práticas adequadas de manejo (RIBAS, 2010). Para o corte verde, o ponto ideal de colheita do sorgo é quando a planta atinge o estádio de emborrachamento ou 50 a 55 dias pós-semeadura. Para pastejo e fenação, esse ponto ideal está entre 0,80 m a 1,00 m de altura (RIBAS, 2010). A

colheita mecânica do grão pode iniciar-se quando a sua umidade estiver entre 20 e 25%. No entanto, o armazenamento do grão deve ocorrer quando estiver com 13% de umidade (FORNASIERI FILHO; FORNASIERI, 2009).

2.2 Selênio e Saúde

Devido ao papel do Se no processo antioxidante, ele contribui para o funcionamento normal do sistema imunológico e tireoidiano (FAIRWEATHER-TAIT et al., 2011), síntese de DNA, e também na reprodução (GUPTA; GUPTA, 2017). Segundo Gupta e Gupta (2017) além das funções citadas, vários estudos relataram: efeito anticancerígeno do Se contra o câncer de fígado, pâncreas, próstata, esôfago e cólon; menor risco de doenças cardiovasculares; implantação de embriões, retenção de placenta, reduz a infertilidade, aumenta a mobilidade do esperma, testosterona e síntese de esperma. Pequenas quantidades de Se são necessárias, mas a ingestão excessiva desse nutriente pode causar toxicidade e gerar problemas de saúde (COZZOLINO, 2000).

O Se é incorporado em selenoproteínas que têm uma ampla gama de efeitos pleiotrópicos (RAYMAN, 2012). Os selenoaminoácidos, selenocisteína (SeCys) e selenometionina (SeMet), são encarregados pela maior parte das ações do Se quando incorporado à dieta (DUMONT et al., 2006), ou seja, essas formas orgânicas de Se que são mais aproveitadas pelo organismo humano ou animal (possuem maior bioacessibilidade). Em contraste com muitos outros micronutrientes, a ingestão de Se varia enormemente em todo o mundo, variando de deficiências a concentrações tóxicas que causam hálito de alho, perda de cabelos e unhas, distúrbios do sistema nervoso. A deficiência de Se causa menor crescimento, afetando o metabolismo ósseo e proporcionando anormalidades na função tireoidiana (REEVES; HOFFMAN, 2009).

Em seres humanos, as funções nutricionais do Se ocorrem por 25 selenoproteínas que têm selenocisteína em seu centro ativo (KRYUKOV et al., 2003). A inserção de selenocisteína para formar selenoproteína é especificada pelo códon UGA no mRNA sob condições típicas (REEVES; HOFFMANN, 2009). Várias selenoproteínas tem o Se como o centro catalítico, como por exemplo, glutationa peroxidase (GSHPx), tiorredoxina redutase e iodotironina-desiodinases, que são relevante na supressão de radicais livres, defesa contra estresse oxidativo, melhoria do sistema imunológico (MÉPLAN, 2011; KAUR, SHARMA, KAUR, 2014). Na baixa provisão de Se, a síntese de algumas selenoproteínas (por exemplo, glutationa peroxidase, GPx4) é priorizada em detrimento de outras (REEVES; HOFFMANN,

2009). Muitas selenoproteínas são enzimas e têm sua importância para a saúde humana demonstrada pelo efeito de polimorfismos de nucleotídeo único (SNPs) em genes de selenoproteína envolvidos no risco de doença ou mortalidade (RAYMAN, 2009).

Segundo Winther et al. (2020) os níveis recomendados de ingestão de Se são 55 µg dia^{-1} pelo Instituto de Medicina dos EUA e 70 µg dia^{-1} pela Autoridade Européia para a Segurança dos Alimentos (EFSA). Em humanos, a deficiência de Se ocorre quando a ingestão alimentar de Se é < 40 µg dia^{-1} e a toxicidade crônica é observada acima dos níveis de > 400 µg dia^{-1} (WINKEL et al., 2012). Na pecuária, a necessidade mínima de Se é de 0,05-0,10 mg kg^{-1} de forragem seca, enquanto a concentração tóxica de Se na alimentação animal é de 2-5 mg kg^{-1} de forragem seca (WU et al., 2015).

O status do Se é medido pelo plasma ou Se sérico (RAYMAN, 2000). O Se plasmático é constituído de cerca de 50% da selenoproteína P que transporta Se entre os tecidos (GUPTA; GUPTA, 2017). Sendo assim o status de Se na população varia de acordo com o país, sendo corresponde à ingestão (RAYMAN, 2000). As ingestões são altas na Venezuela, no Canadá, nos EUA e no Japão, e por outro lado, são menores na Europa, particularmente na Europa Oriental. A China tem áreas de deficiência e excesso de Se. A deficiência de Se ocorre em áreas onde o seu teor no solo é baixo, incluindo partes da Europa, China, América do Norte, Austrália, Nova Zelândia e África do Sul (SORS, ELLIS, SALT, 2005). As ingestões na Nova Zelândia, que antes eram baixas, melhoraram após o aumento da importação de trigo australiano com Se alto (RAYMAN, 2008). As recomendações para a ingestão de Se neste país são em média 60 µg dia^{-1} para homens e 53 µg dia^{-1} para mulheres (RAYMAN, 2004).

A toxicidade do Se ocorre em áreas onde o Se no solo é naturalmente alto, incluindo áreas da China, Índia e Estados Unidos. A toxicidade de Se de ocorrência natural pode ser exacerbada pela irrigação de solo selenífero, mineração e uso de combustíveis fósseis ricos em Se (TERRY et al., 2000). Em comparação com outros elementos, o limiar entre o benefício e a toxicidade é pequeno para a Se.

Há plantas que quando em solos seleníferos são tolerantes ao Se, e acumulam o Se em altas concentrações, mas a maioria das plantas são sensíveis ao Se, e acumulam o Se em concentrações muito baixas (TERRY et al., 2000). Razões para a variabilidade na ingestão referem-se não só ao teor de Se do solo onde são cultivadas as plantas, mas também a fatores que determinam a disponibilidade de Se na cadeia alimentar, incluindo especiação de Se, pH do solo, teor de matéria orgânica e presença de íons que podem se complexar com o Se (JOHNSON; FORDYCE; RAYMAN, 2010).

2.3 Selênio no Solo

Nos solos de todo o mundo são estimados teores médios de Se variando entre 0,05 a 1,5 mg kg^{-1} (KABATA-PENDIAS; PENDIAS, 2001). No entanto, há solos com teores elevados de Se variando de >2 a 5000 µg kg^{-1} (HARTIKAINEN, 2005). Os solos podem ser classificados quanto aos teores de Se que apresentam. Solos com teores de Se <100 µg kg^{-1} são considerados deficientes (FORDYCE, 2007). O teor de Se no solo sofre grande variação em função da quantidade de Se do material origem e do tipo de solo. O selênio é originário de rochas sedimentares constituídas no decorrer do período carbonífero ao quaternário (WHITE et al., 2004). A ocorrência de Se no solo também é dependente da matéria orgânica e precipitação (SORS; ELLIS; SALT, 2005).

As reservas mundiais de Se podem ser encontradas nos países: Peru, China, Chile, Estados Unidos, Canadá, Zâmbia, Filipinas, Zaire, Austrália e Nova Guiné (LIU et al., 2011). Mesmo a China que é classificada em quarto lugar em reservas de Se em todo o mundo, encontra-se a deficiência de Se em um cinturão geográfico com baixos teores de Se, o qual atinge 71,2% de terras chinesas (ZHU et al., 2009). Nos solos de clima tropical, onde o intemperismo é mais intenso, os teores de Se são muito baixos (COMBS, 2001; KABATA-PENDIAS; PENDIAS, 2001) devido a esses solos serem ricos em óxidos de ferro e alumínio na fração argila. A adsorção de Se é maior com o aumento do teor de argila, consequentemente há redução na disponibilidade de Se para as plantas (ABREU et al., 2011).

Araújo et al. (2019) ao estudarem a adsorção de selenito em solos tropicais do Cerrado Brasileiro observaram que todos os solos apresentam alta adsorção de selenito, principalmente os argilosos. Os solos arenosos, tiveram maiores quantidades adsorvidas de selenito em solos não cultivados. Segundo os autores, o principal mecanismo de adsorção de selenito em solos oxídicos é pelo complexo de esfera interna, o que explica as baixas quantidades de selenito dessorvidas encontradas. Ou seja, o selenito é muito mais retido em solos tropicais e menos disponível para as plantas do que o selenato (ARAÚJO et al. 2020). Áreas deficientes em Se são comumente relatadas no Brasil (ZHU et al., 2009; YIN et al., 2012), porém o Estado do Pará na Amazônia brasileira (LEMIRE et al., 2009) foi considerado uma região rica em Se.

No Brasil, ainda são poucos os estudos de teor e comportamento de Se em solos (ARAÚJO et al. 2019, 2020; CARVALHO et al, 2019; GABOS; ALLEONI; ABREU, 2014; LESSA et al., 2016; SHALTOUT et al., 2011, SILVA JUNIOR et al., 2017; SANTOS et al. 2021). Em estudos realizados em São Paulo, Gabos, Alleoni e Abreu (2014) relataram concentrações médias de Se de 93 µg kg^{-1} para camadas superficiais e 127 µg kg^{-1} para

amostras subsuperficiais de solo em áreas agrícolas. Shaltout et al. (2011) encontraram concentrações de Se na camada superficial de 604 µg kg^{-1} no Amazonas, 113 µg kg^{-1} no Mato Grosso do Sul, 419 µg kg^{-1} no Pará, 248 µg kg^{-1} no Rio Grande do Sul, 262 µg kg^{-1} no Santa Catarina, 1692 µg kg^{-1} no Minas Gerais, 599 µg kg^{-1} no Ceará, 215 µg kg^{-1} no Goiás e 370 µg kg^{-1} no Paraná. Silva Junior et al. (2017) encontraram uma variação <65,76 a 625,91 µg kg^{-1} na concentração total de Se no solo no bioma da Amazônia (Acre, Mato Grosso, Amazonas, Roraima e Amapá) em áreas com castanheira.

O selênio no solo existe em várias formas inorgânicas, como Se elementar, seleneto, selenito, selenato e em formas orgânicas. As quantidades de cada forma de Se na solução do solo são regidas por diferentes fatores, como por exemplo, pH, potencial de oxidação e processos biológicos (JEŽEK et al., 2012; KABATA-PENDIAS; MUKHERJEE, 2007). Um exemplo da interferência da acidez do solo no acúmulo de Se foi relatado por Silva Junior et al. (2017) em castanha-do-brasil. Os autores observaram que o acúmulo de Se em castanheira foi maior em solos com maior teor de Se total, mas diminuiu sob condições ácidas do solo, demonstrando a importância desta propriedade do solo para a retenção de Se e disponibilidade para as plantas.

Já, Lessa et al. (2016) observaram o efeito da textura do solo na adsorção de Se nos solos, uma vez que foi encontrada maior retenção de Se nos solos argilosos, quando comparados aos solos arenosos. O que evidenciou a importância dessa característica física do solo sobre o comportamento do Se no solo. Depois os estudos de Araújo et al. (2019 e 2020), bem como de Santos et al. (2021) observaram a influência de ânions competidores na retenção e disponibilidade de Se (como selenato e selenito).

Araújo et al. (2020) avaliaram o comportamento de sorção de selenato e selenito em diferentes camadas de solo tropical tratado com diferentes doses de gesso agrícola. Estes autores observaram que a adsorção de selenato aumentou com a profundidade do solo e diminuiu com o aumento dos teores de sulfato no solo. Por outro lado, o selenito foi adsorvido consistentemente em teores mais elevados, quando comparado com o selenato, em qualquer profundidade do solo e seu comportamento de adsorção não foi afetado pela presença de sulfato. Além disso, o selenito foi menos dessorvido do que o selenato em todas as condições.

Em solos onde há condições favoráveis ao crescimento das plantas o Se pode ser encontrado com predominância das formas de selenito e selenato. Quando o solo apresenta pH ácido e alta umidade o selenito é a espécie química predominante (NAKAMARU; TAGAMI; UCHIDA, 2005; NEAL, 1995). Entretanto, em solos pH neutro ou alcalino, o selenito tende a oxidar formando selenato (GEERING et al., 1968). Em solos agrícolas a

forma predominante de Se é o selenato, o qual é mais solúvel em água do que o selenito (SORS; ELLIS; SALT, 2005). Segundo Lessa et al. (2016) o fornecimento de Se por meio de selenato nos solos é dependente do manejo do solo devido a mobilidade desse elemento que é maior em solos cultivados, quando comparado a solos não cultivados, favorecendo sua absorção pelas plantas.

Assim, a forma química do Se no solo tem efeito sobre várias propriedades, dentre elas, a mobilidade e disponibilidade para as plantas (KOPSELL; KOPSELL, 2007). As formas de Se também se diferenciam em relação a absorção e mobilidade dentro da planta (LI; MCGRATH; ZHAO, 2008). As espécies de plantas apresentam capacidade diferenciada de concentrar e acumular Se, como por exemplo, na distribuição de Se em partes da planta, e a capacidade de absorver Se ao invés do enxofre (S) (SCHIAVON; PILON-SMITS, 2017).

O fornecimento de outros nutrientes durante o cultivo do solo pode afetar a mobilidade do Se. Segundo Lessa et al. (2016) a mobilidade é maior em solos cultivados e assim, o fornecimento depende do manejo do solo adotado anteriormente. Lessa et al. (2016) estudaram a adsorção e dessorção de Se (selenato) em solos brasileiros do bioma Cerrado, com amostras de solo coletadas em solos cultivados e não cultivados, compreendendo solos classificados de acordo como sua caracterização física como argilosos e arenosos.

Os teores de Se adsorvidos foram maiores nos solos não cultivados, quando comparados aos solos cultivados e a adsorção de Se foi maior nos solos argilosos em relação aos solos arenosos. Sendo assim esses autores afirmam que a adição de selenato ao solo é uma boa estratégia para aumentar os níveis de Se em culturas, principalmente quando as culturas são cultivadas em solos que foram cultivados ao longo do tempo devido à sua baixa capacidade de adsorção de Se (alta disponibilidade de Se) (LESSA et al., 2016).

Santos et al. (2021) adicionaram fontes de P e S em solos enriquecidos com Se para verificar o potencial de dessorção, e aferiram com o plantio de capim Mombaça. Em todas as doses de selenato adicionadas no solo argiloso, os teores de Se na matéria seca da parte aérea do capim Mombaça aumentaram com o aumento da taxa de adubação com P, concordando com os resultados de dessorção. Estes autores avaliaram a dessorção de Se em solos tropicais por sulfato e fosfato sob taxas crescentes de adubação fosfatada, bem como, os efeitos de fosfato e sulfato como ânions competitivos na dessorção de Se. A adsorção de Se variou de acordo com a especiação de Se, teor de Se e textura do solo. O selenito apresentou maior adsorção que o selenato. Em geral, as quantidades de selenato dessorvidas aumentaram com o aumento da adição de P e S. Porém, apenas o P teve um efeito positivo na liberação de selenito dos solos.

2.4 Selênio em Plantas

Os solos quando apresentam concentrações de Se consideradas baixas são responsáveis por teores baixos nos solos podendo resultar em baixos teores nas plantas e, desse modo repercutindo em ingestão de Se em quantidades baixas por humanos e animais que por ventura se alimentam dessas plantas (DURÁN et al., 2013). Ainda não se obtiveram indícios de que o Se seja essencial em plantas superiores (TERRY et al., 2000). Embora não seja essencial para plantas superiores, o Se é considerado um elemento benéfico, estimulando o crescimento quando em baixos níveis (PILON-SMITS et al., 2009).

As plantas absorvem e assimilam o Se em aminoácidos como a selenometionina e selenocisteína pela mesma via em que absorvem e assimilam o S (TERRY et al., 2000). A absorção, translocação e distribuição de Se pelas plantas são uma sequência de eventos resultantes da combinação de espécie vegetal, fase de desenvolvimento, forma de Se, dose de Se, condições fisiológicas, e existência de outras substâncias (LI; MCGRATH; ZHAO, 2008; RENKEMA et al., 2012). Sendo que a translocação de um íon ou molécula pelo tecido vegetal depende da taxa de carregamento do xilema e também da taxa de transpiração (RENKEMA et al., 2012).

As raízes das plantas absorvem as formas inorgânicas (selenato e selenito) e orgânicas do Se (selenometionina e selenocisteína) dos solos, porém não absorvem o Se elementar (KÖNIG MUTHURAMALINGAM; DIETZ, 2012). A absorção de Se nas plantas é intermediada por transportadores existentes na membrana das células da raiz. Sendo que, o selenito é transportado pelo mesmo mecanismo de transporte de fosfato (LI; MCGRATH; ZHAO, 2008), enquanto o selenato é transportado pelos transportadores e canais de sulfato (WHITE et al., 2004). Segundo Hartikainen (2005), a forma inorgânica, selenato (SeO_4^{2-}), é a mais solúvel em água, móvel e biodisponível em solos de óxidos, com baixa afinidade de adsorção por superfícies de óxidos.

Após a absorção, inicialmente o Se é convertido em selenito (GUPTA; GUPTA, 2017). Sendo que ocorre o transporte do selenato para os cloroplastos, no qual é processado pela via de assimilação do S (HAWKESFORD, 2005). Nesta fase, é necessária a atividade sequenciada das enzimas ATP sulfurilase e adenosina-5-fosfossulfato redutase. O ATP sulfurilase catalisa a hidrólise do ATP para formar adenosina-5-fosfosselenato (PILON-SMITS; QUINN, 2010), o qual é em seguida reduzido a selenito pela adenosina-5-fosfossulfato redutase (SORS; ELLIS; SALT, 2005). Posteriormente, o selenito é transformado em seleneto por meio da ação da enzima sulfito redutase (GONZÁLEZ-

MORALES et al., 2017). Em plantas, esta etapa também pode ser reduzida pela glutationa ou glutaredoxinas (WALLENBERG et al., 2010) por meio de um processo de redução não enzimática (FREEMAN et al., 2010; MEHDI et al., 2013). Em seguida, o seleneto é incorporado a aminoácidos, como por exemplo selenocisteína e selenometionina, originando assim os selenoaminoácidos (FREEMAN et al., 2010).

O seleneto quando convertido em selenocisteína é devido a ligação com O-acetilserina (OAS) na presença da enzima cisteína-sintetase para produzir selenocisteína (PILON-SMITS; QUINN, 2010), uma vez que a cisteína-sintase tem maior afinidade para o seleneto quando comparado ao sulfeto (GUPTA; GUPTA, 2017). A transformação de selenocisteína em selenometionina ocorre em um processo sequencial. Preliminarmente, a selenocistationina é formada pela cistationina γ-sintase e, logo após, a selenohomocisteína recebe a ação da cistationina β-liase. Por fim, a selenohomocisteína é alterada e forma a selenometionina pela ação da metionina sintase no citosol (PILON-SMITS; QUINN, 2010).

Fatores como a espécie da planta e condições ambientais podem influenciar a transformação da selenocisteína em Se elementar quando na presença da enzima selenocisteínaliase, ou também pode ser metilado em metil-selenocisteína pela selenocisteína metiltransferase, ou ainda ser convertido em selenometionina por várias enzimas (GUPTA; GUPTA, 2017). Tanto o selenocisteína quanto a selenometionina podem ter diversas finalidades nas plantas. Além da incorporação em proteínas, elas podem ser metilados em formas voláteis ou transformadas em aminoácidos não proteicos (WHITE, 2018).

A translocação de Se da raiz para as folhas depende da forma de Se. O selenato é mais facilmente transportado do que o selenito, ou selenometionina (TERRY et al., 2000). O Se orgânico no solo ocorre em complexos com matéria orgânica e combinado com coloides orgânicos ou organo-minerais. Compostos organo-Se incluem os Se-aminoácidos metilados ou não metilados e formas voláteis (dimetil seleneto, dimetil disseleneto) (WINKEL et al., 2015). A metilação da selenocisteína impossibilita a integração em proteínas não específicas (WANG; BOCK; NEUHIER, 1999). Segundo Freeman et al. (2010), a selenometiltransferase é uma enzima relevante nos processos de hiperacumulação e tolerância ao Se.

Em função da quantidade de Se acumulado no interior das células pode-se classificar as plantas como: hiperacumuladoras, acumuladoras secundárias e não acumuladoras (BODNAR; KONIECZKA; NAMIESNIK, 2012). As plantas hiperacumuladoras podem conter quantidades de Se em seus tecidos maiores que 1000 mg kg^{-1} de peso seco. Estas plantas crescem tipicamente em solos seleníferos (TERRY et al., 2000). As plantas classificadas como acumuladoras secundárias podem acumular o Se em seus tecidos e não

expor sinais de toxicidade numa faixa de 100-1000 mg kg^{-1} de peso seco (GUPTA; GUPTA, 2017). A maior parte das plantas se encaixam na classificação de não acumuladoras, ou seja, não acumulam o Se em seus tecidos em quantidade superior a 100 mg kg^{-1} de peso seco (WHITE et al., 2004). Quando essas espécies de plantas crescem em solos ricos com Se apresentam crescimento inferior ou morrem (BODNAR; KONIECZKA; NAMIESNIK 2012).

Em comparação com a maioria dos elementos, o limiar entre o benefício e a toxicidade é pequeno para o Se. Tanto a deficiência quanto a toxicidade de Se são problemas em todo o mundo. Algumas espécies de plantas quando crescem em solos seleníferos são tolerantes ao Se, e acumulam este elemento em altas concentrações, mas a maioria das plantas são sensíveis ao Se, e acumulam este elemento em concentrações muito baixas (TERRY et al., 2000).

Em níveis elevados, o Se é tóxico para a maioria das plantas, devido à incorporação inespecífica de Se em compostos de S como por exemplo a incorporação de selenocisteína ou selenometionina em proteínas e ao estresse oxidativo (VAN HOEWYK, 2013) que ocorre provavelmente pelo desequilíbrio nos níveis de GSH, ferredoxinas, grupos tiol e NADPH nas células (HARTIKAINEN, 2005).

O estresse oxidativo pode provocar a disfunção do DNA, proteínas e lipídios nas células quando a produção de espécies reativas de oxigênio (EROs) é maior do que sua erradicação (MITTLER, 2002). No nível de fitotoxicidade, o Se atua como pró-oxidante aumentando a concentração de peróxido de hidrogênio (H_2O_2) e induzindo a peroxidação lipídica (RÍOS et al., 2008) e pode ocorrer por meio do uso de altas doses ou tempo de exposição excessivo (HAWRYLAK-NOWAK; MATRASZEC; POGORZELEC, 2015).

O acúmulo de selenoproteínas nas plantas pode resultar na geração de EROs (VAN HOEWYK, 2013). O crescimento da planta pode ser afetado pelas EROs em conjunto com hormônios como ácido salicílico, ácido jasmônico, giberelina, ácido abscísico, etileno e auxina (MHAMDI; VAN BREUSEGEM, 2018). Quando em doses adequadas, o Se pode promover efeitos benéficos. Segundo Lessa et al. 2020, a adição de Se influenciou positivamente a atividade das enzimas antioxidantes (SOD, CAT e APX), embora não tenha aumentado a produção de grãos de arroz. Os mecanismos que causam os efeitos benéficos ainda são desconhecidos, mas podem estar associados à atividade antioxidante aumentada (HARTIKAINEN, 2005).

FENG, WEI e TU (2013) descreveram os efeitos benéficos do Se no crescimento, desenvolvimento e funcionamento das plantas. Principalmente quando o Se é fornecido em baixas doses favorece resistência das plantas a vários estresses abióticos (FENG; WEI; TU, 2013) a estresse hídrico (NAWAZ et al., 2015; FENG; WEI; TU, 2013; ANDRADE et al.,

2018; RAVELLO et al., 2021), altas temperaturas (DJANAGUIRAMAN; PRASAD; SEPPANEN, 2010; HASANUZZAMAN et al., 2014); em radiação UV-B (YAO et al., 2013); baixas temperaturas (CHU; YAO; ZHANG, 2010; KUMAR et al., 2012) Djanaguiraman, Prasad e Seppanen (2010) observaram efeito positivo na fotossíntese em plantas de sorgo, o que provavelmente pode ter ocorrido em função da diminuição dos níveis de EROs e aumento da atividade antioxidante na aplicação de baixas doses de Se.

2.5 Biofortificação com Selênio

As alternativas para melhorar a ingestão de Se por humanos e animais foram estudadas por vários pesquisadores (EUROLA et al., 1991; BROADLEY et al., 2010; SEPPÄNEN et al., 2010; BOLDRIN et al., 2012, 2013, 2016; DURAN et al., 2013; BAÑUELOS; LIN; BROADLEY, 2015; ANDRADE et al, 2018; LARA et al. 2019; LESSA et al., 2019, 2020). A ingestão dietética de Se pode ser complementada pelo uso direto de suplementos contendo Se (BROADLEY et al., 2006) ou pela biofortificação de alimentos, incluindo fertilização e abordagens genéticas (WHITE; BROADLEY, 2009). A biofortificação agronômica pode ser utilizada para produzir alimentos enriquecidos com Se e assim reduzir as deficiências desse elemento, uma vez que ocorre em todas as regiões do mundo (BROADLEY et al., 2006, 2010) e áreas com deficiência de Se são mais comuns do que áreas com concentrações de Se consideradas alta (HAUG et al., 2007). Uma das questões-chave na biofortificação é selecionar o método mais apropriado para biofortificar plantas.

A maneira mais viável de introduzir Se de forma gradual e segura na cadeia alimentar é usar fertilizantes inorgânicos de Se (LYONS et al., 2004; LI et al., 2007). Ros et al. (2016) mostraram que os fertilizantes à base de selenato têm um alto potencial para aumentar a captação de Se pelas culturas e, consequentemente, a ingestão de Se em animais e humanos. Lessa et al. (2020) observaram que a biofortificação agronômica com Se (selenato via solo, selenato via foliar, e selenito via foliar) foi eficaz na melhoria da qualidade nutricional dos grãos de arroz em relação ao teor de Se, sendo necessária menor dose de Se para atingir a ingestão recomendada de Se com a aplicação de Se via folha em comparação com a aplicação via solo, considerando solos tropicais brasileiros. No entanto, os estudos sobre o fornecimento de Se orgânico ainda são incipientes. A biofortificação agronômica de culturas alimentares foi praticada comercialmente em regiões deficientes em Se adicionando fertilizantes inorgânicos modificados com Se aos solos na Finlândia (ALFTHAN et al., 2011).

A forma mais comum de adição de Se usada é o selenato e, em menor grau, selenito, e

como sais de sódio ou bário, podem ser aplicados em formas granulares diretamente no solo, ou como líquido em alto volume (BROADLEY et al., 2010). A aplicação de grandes quantidades de fertilizantes com Se pode não é uma estratégia sustentável devido ao potencial de lixiviação do excesso de Se. Outra desvantagem é a necessidade de aplicações regulares, o que pode tornar essa abordagem onerosa (WHITE; BROADLEY, 2009).

O enriquecimento efetivo de culturas agrícolas com Se usando fertilizantes enriquecidos em Se no solo pode ser desafiador devido à variação de parâmetros como: concentrações de Se no solo, tipo de solo, potencial redox, pH e atividade microbiológica (HARTFIEL; BAHNERS, 1988). Como alternativa, a aplicação foliar de Se tem sido usada para enriquecer o Se em produtos agrícolas (SMRKOLJ et al., 2006). Com este método, uma solução contendo Se é pulverizada na superfície da folha.

Como a química do solo e os processos microbiológicos têm menor impacto sobre o Se, garante-se maior eficácia de absorção com a aplicação de baixos volumes de solução contendo Se. Broadley et al. (2010) e Schiavon et al. (2016) relataram a eficácia da biofortificação foliar com Se. Fatores como a quantidade de Se aplicada, a área foliar, a estrutura da superfície da folha e as diferenças no metabolismo específico da planta diferem entre as culturas e devem ser considerados (BAÑUELOS; LIN; BROADLEY, 2017).

Os efeitos da biofortificação com Se foram observados em várias culturas, como: arroz (*Oryza sativa* L.) (BOLDRIN et al., 2012, 2013; DENG et al., 2017; ANDRADE et al., 2018; HUANG et al., 2018; LESSA et al., 2019, 2020; AHMAD et al., 2021), trigo (*Triticum aestivum* L.) (GALINHA et al., 2014; NAWAZ et al., 2015; BOLDRIN et al., 2016; LARA et al., 2019; RAMKISSOON et al., 2019), feijão (*Phaseolus vulgaris*) (RAVELLO et al., 2021), milho (*Zea mays* L.) (WANG et al., 2019; LONGCHAMP et al., 2015), sorgo (*Sorghum bicolor* L.) (QURESHI et al., 2021), leucena (*Leucaena leucocephala* (Lam.) de Wit) (ÁVILA et al., 2020), alho-poró (*Allium ampelop*rasum L.) (LAVU et al., 2012), cebola (*Allium cepa* L.) (KÁPOLNA et al., 2012), cenoura (*Daucus carota* L.) (OLIVEIRA et al., 2018), rabanete (*Raphanus sativus* L.) (SCHIAVON et al., 2016; SILVA et al., 2020a, 2020b; CIPRIANO et al., 2022), batata (*Solanum tuberosum* L.) (OLIVEIRA et al., 2019; ZHANG et al., 2019; ZHANG et al., 2021), brócolis (*Brassica oleracea* L. var. Itálica) (RAMOS et al., 2011; ÁVILA et al., 2013), rúcula (*Eruca sativa* Mill.) (SANTIAGO et al., 2020), alface (*Lactuca sativa* L.) (RAMOS et al., 2011; SANTIAGO et al., 2020), mirtilo (*Vaccinium* spp) (LI et al., 2018), maçã (*Malus domestica*) (GROTH et al., 2021), morango (Fragaria x ananassa Duch.) (SANTIAGO et al., 2018).

3 CONSIDERAÇÕES FINAIS

A biofortificação agronômica é uma técnica importante para o fornecimento de elementos essenciais tanto para humanos quanto para animais. O fornecimento de Se para plantas tem sido estudado em várias culturas de interesse econômico, como por exemplo arroz e trigo, mas os estudos com sorgo ainda são incipientes. O sorgo vem ganhando destaque na agricultura brasileira por tolerar baixos índices pluviométricos e altas temperaturas, o que faz seu cultivo adequado na "safrinha". O sorgo apresenta menor custo de produção e valor nutritivo semelhante quando comparado ao milho.

No Brasil, o sorgo é principalmente utilizado na alimentação de animais como aves, suínos e bovinos. Os benefícios do Se para animais são: melhora da performance produtiva, aumento da resistência a doenças e também melhoria na qualidade dos produtos de origem animal. Uma vez que o Se é um elemento insuficiente na dieta animal, a biofortificação do sorgo seria uma forma eficiente de fornecer esses nutrientes para os animais.

A partir deste estudo novas pesquisas poderão ser realizadas com objetivos de verificar os efeitos do Se em outros tipos de sorgo como por exemplo no sorgo utilizado para silagem, identificação da melhor combinação entre doses, fontes e modo de aplicação visando maior produção, melhora na qualidade do produto final, melhor aproveitamento do nutriente evitando perdas e contaminação do meio ambiente. Além disso, melhora da tolerância da planta a stress ambientais, como seca, baixas e altas temperaturas.

Neste contexto é de suma importância a realização de estudos visando aspectos agronômicos, fisiológicos e bioquímicos e a influência do ambiente em plantas de sorgo biofortificadas com Se. A adoção de estratégias de fertilização adequadas é relevante para possibilitar a absorção de Se e limitar possíveis riscos de contaminação ambiental. Além disso, as reservas globais de Se são poucas e com a baixa recuperação dos fertilizantes de Se, o que faz com que exista a inquietação sobre a possibilidade dessas reservas se findar.

Ao optar por um método de adubação adequada que forneça Se, é primordial conhecer a ciclagem do Se nos solos e a ação desse elemento nas plantas. Sendo assim, é importante pesquisar os efeitos de fertilizantes contendo Se nos agroecossistemas por meio de técnicas adequadas para avaliar as interações entre propriedades do solo, condições climáticas, sistemas de cultivo e manejo de fertilizantes. Os resíduos vegetais enriquecidos com Se podem ser uma fonte de Se para as plantas e reciclar Se nos agroecossistemas, reduzindo assim a quantidade necessária desse elemento. Portanto essas informações como estas podem auxiliar o agricultor a introduzir a biofortificação para quaisquer condições locais.

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SEGUNDA PARTE

ARTIGO 1 – SELENIUM BIOFORTIFICATION VIA SOIL AND ITS EFFECT ON PLANT METABOLISM AND MINERAL CONTENT OF SORGHUM PLANTS

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SELENIUM BIOFORTIFICATION VIA SOIL AND ITS EFFECT ON PLANT METABOLISM AND MINERAL CONTENT OF SORGHUM PLANTS

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Abstract

Sorghum has excellent potential to be used in the supplementation of selenium (Se) to humans and animals when compared with other grain crops due to its protein content, rusticity, and great tolerance to various stressing conditions. Few studies have been dedicated to explore Se biofortification strategies in sorghum plants, especially in tropical agroecosystems. In this context, the objective of this work was to evaluate the efficiency of different Se doses (0, 60, 240, and 480 µg dm^{-3}) and Se sources (SeA - potassium hydroxy-selenide, SeB - methyl hydroxy-selenide, SeC - hydroxy-selenide, and SeD - sodium selenate) in Se biofortification of *Sorghum bicolor* L., as well as in its antioxidant system, and the contents of carbohydrate and mineral in response to soil Se application. The increase in the Se content in the grains and the shoots were closely to the increments in applied Se doses. The greater absorption efficiency by the roots and % Se recovery occurred with the application of SeD. Selenium application influenced both the antioxidant system and carbohydrates in a positive way. In summary, considering the parameters evaluated, the Se sources SeB (methyl hydroxy-selenide) and SeC (hydroxy-selenide) at a dose of 240 µg Se dm^{-3} and SeD (sodium selenate) at a dose of 60 µg Se dm^{-3} are considered promising for the biofortification of sorghum.

Keywords: antioxidant enzymes, food composition, selenate, selenide, agronomic biofortification.

1. Introduction

Selenium (Se) is an essential element for humans and animals, and its absence in food is a prevailing problem worldwide (Schiavon et al., 2020). The relevance of this element is due to its antioxidant and anti-cancer properties, which made this nutrient to be recognized as essential for human health (Thiry et al., 2012). Selenium is co-translationally incorporated into the polypeptide chain by integrating the 21st naturally occurring amino acid, selenocysteine, encoded by the UGA codon (Zoidis et al., 2018), activating the antioxidant system of the organisms (e.g., plants, animals, and humans) (Ravello et al., 2021).

Because of its recognized benefits, Se has recently been studied for use against the coronavirus pandemic, especially considering its potential to strengthen the human immune system (Kieliszek and Lipinski, 2020). According to Winther et al. (2020) recommended levels of Se intake are 55 μg day^{-1} by the US Institute of Medicine and 70 μg day^{-1} by the European Food Safety Authority (EFSA). In humans, Se deficiency occurs when the dietary Se intake is < 40 μg day^{-1} and chronic toxicity was observed above levels of > 400 μg day^{-1} (WINKEL et al., 2012).

Animals, such as ruminants, are naturally more susceptible to Se deficiency as a consequence of the specificities of the rumen fermentation process. The microbial activity forms insoluble substances, and consequently the loss of Se due to its use by ruminal microorganisms. The microbiota also converts inorganic forms of Se into organic compounds such as selenoaminoacid, mainly selenomethionine (Prauchner, 2014). The National Research Council - NRC (2001) established the Se recommendation for dairy cattle of 0.3 mg kg^{-1} dry mass (DM) based diet. For beef cattle, broiler chicken, and swine, Se recommendations are 0.1 to 0.3, 0.15, and 0.30 mg kg^{-1} of diet (NCR, 1996, 1994, 1998), respectively.

The immune system depends on a set of specific selenoproteins that contain selenocysteine at their active sites. The complete expression and enzymatic activity of Se depend on the abundant Se supply (Schomburg, 2020). Regarding plants, Se essentiality has not yet been confirmed. However, beneficial effects occurred at appropriate concentrations, such as: increased productivity (Boldrin et al., 2013); accumulation of biomass (Lara et al., 2019); higher activity of antioxidant enzymes (Feng et al., 2016, Ravello et al., 2021), photosynthetic rate (Andrade et al., 2018), carbohydrate content (Lara et al., 2019), and delayed some of the effects of senescence and may improved plants use of short-wavelength light by plants (Hartikainen, 2005). Moreover, the experience of Finnish soils with low Se content provides evidence that the supplementation of commercial fertilizers with sodium

selenate had a positive impact on Se transfer from the soil to plants, animals, and humans (Hartikainen, 2005).

Selenium can be found in inorganic and organic forms in soils, with different oxidation states ranging from -2 to +6 (Schiavon et al., 2020). It can exist in the oxidation states of -2 (selenide Se^{2-}), 0 (elemental Se), +4 (selenite SeO_3^{2-}) and +6 (selenate SeO_4^{2-}) (Broadley et al., 2012). The inorganic form, selenate, is the most soluble in water, mobile, and bioavailable in oxidic soils, due to its smaller adsorption affinity for oxide surfaces, when compared with selenite (Araújo et al., 2020; Lopes et al., 2017; Santos et al., 2021;). Moreover, organic Se in the soil occurs in complexes with organic matter and combined with organic colloids or organo-minerals. Organo-Se compounds include methylated or unmethylated Se-amino acids and volatile Se forms (dimethyl selenide, dimethyl diselenide) (Winkel et al., 2015). Selenium forms potassium hydroxy-selenide, methyl hydroxy-selenide, and hydroxy-selenide are examples of compounds that can be formed with Se.

Understanding Se interactions in the soil is essential as this will ensure better absorption and accumulation of this element by plants. Enriching plant-based foods with Se is an excellent approach for providing Se to the population since cereal grains are important staples in developing countries due to their high consumption (Khanam and Platel, 2016). In addition to Se, elements such as Ca, Mg, Cu, Fe, Zn, and I are frequently observed at deficient levels in the diets (White and Broadley, 2005). Insufficient mineral intake can affect mental and physical development, limiting work performance and contributing to morbidity due to infections (Hussain et al., 2010; Ng'uni et al., 2011). Indeed, several studies have shown that adequate mineral content in wheat, maize, and sorghum (Hussain et al., 2010; Ndukwe et al., 2015; Ng'uni et al., 2011) is key for populations worldwide, contributing to a balanced diet.

Sorghum has significant variation regarding its use in each region of the world, being used in human nutrition in Africa and Asia; whereas in the Americas and Australia, it is used mostly as a component of animal feed and ethanol production (Paiva et al., 2017). The progressive introduction of sorghum in human nutrition is mainly due to the absence of gluten in its composition (Pontieri et al., 2013), combined with characteristics such as the cholesterol-lowering effect, anti-inflammatory action, slow digestibility, and blocking the expansion of cancer cells in the esophagus and human colon (Awika et al., 2009), besides the fact that the sorghum plant itself is very rustic and tolerant to a number of stressing conditions (Adebowale et al., 2020).

Cereal crops were commonly used in biofortification programs as they form the basis

of several diets (Taylor et al., 2012), so various strategies have been developed to increase the levels of essential minerals in grain crops through agronomic interventions and genetic selection (White and Broadley, 2005). Researchers have shown promising results for sorghum grain biofortification with Fe and Zn levels (Ashok Kumar et al., 2013), yet studies aiming to demonstrate the efficiency of agronomic biofortification of sorghum with Se are still scarce, mainly in tropical agroecosystems. One exception is the work developed by Qureshi et al. (2021), who studied sorghum biofortification with Fe, Zn, and Se, and reported that these elements increased the growth and quality of different sorghum accessions used as forage, with Se providing greater plant height, stem diameter, and 1000 grains weight.

Still, to the best of our knowledge, there are no studies so far demonstrating the response of sorghum plants to biofortification with Se, especially concerning the most efficient Se sources and the most adequate Se doses. The success of biofortification to enrich plants with Se depends on several factors, such as the Se source, the application mode, the crop, among others. In this context, the objective of this work was to evaluate the efficiency of different doses and sources of Se in the biofortification of *Sorghum bicolor* L., as well as in its antioxidant system, and the contents of carbohydrates and mineral contents in response to soil Se applications.

2. Material and Methods

2.1. Experimental Design

An experiment was carried out between August and December 2018, in which the cultivar BM 737 (*Sorghum bicolor* (L.) Moench) was used to evaluate the effects of Se biofortification on sorghum plants. This cultivar has as main characteristics: 1.35 to 1.45 m of plant height; 51 to 55 days from sowing to flowering; early cycle, from 120 to 130 days from sowing to harvest; upright leaf architecture; medium grains, red and without tannin; high resistance to diseases such as rust and anthracnose; recommended for grain production and wet grain silage.

The experiment was conducted under greenhouse conditions in a completely randomized design, using a 4 x 2 + 2 factorial scheme with four Se sources (SeA - potassium hydroxy-selenide, SeB - methyl hydroxy-selenide, SeC - hydroxy-selenide, and SeD - sodium selenate), two Se doses (60 and 240 µg dm^{-3}), and two extra treatments (hereafter called "control" - without Se application - and "additional" - with Se application of 480 µg dm^{-3} using sodium selenate) with four replicates each, totaling 40 experimental plots. Selenium

sources SeA, SeB, and SeC are potassium hydroxy-selenide, methyl hydroxy-selenide, and hydroxy-selenide, containing 26, 38, and 40% of Se in their composition, respectively. Selenium doses were chosen based on previous experiments performed by our research group with grain crops (Boldrin et al., 2012; 2013; Ravello et al., 2021).

2.2. Cultivation conditions

The pots were filled with 5 dm^3 of dystrophic Red-Yellow Latosol (Santos et al., 2018), with a sandy-loam texture corresponding to the Typic Haplustox in Soil Taxonomy (Soil Survey Staff, 2014). Initially, the soil was air-dried and passed through a 4-mm sieve. Then, it was characterized in terms of its physical and chemical properties and the total oxide content, according to Teixeira et al. (2017) (Supplementary material Table S1).

Liming was performed to increase base saturation to 70% using $CaCO_3$ and $MgCO_3$ in a 3:1 ratio. After 30 days of incubation with soil humidity close to 60% of the total pore volume, a base fertilization was performed, which consisted of: 80 mg of N, 140 mg of P, 90 mg of K, 50 mg of S, 0.5 mg B, 1.5 mg of Cu, 0.1 mg of Mo and 5.0 mg of Zn per dm^{-3} of soil. It was used the following sources: potassium phosphate monobasic (KH_2PO_4) ammonium nitrate (NH_4NO_3), magnesium sulfate ($MgSO_4.7H_2O$), zinc sulfate ($ZnSO_4.7H_2O$), copper sulfate ($CuSO_4.5H_2O$), manganese sulfate ($MnSO_4.H_2O$), boric acid (H_3BO_3) and ammonium molybdate ($[NH_4]_6Mo_7O_{24}.4H_2O$).

Next, ten sorghum seeds were planted per pot. Ten days after seedling emergence, thinning was done, leaving one seedling per pot. During sorghum cultivation, additional fertilization with 110 mg of P dm^{-3}, 200 mg of N dm^{-3} and 110 mg of K dm^{-3} of soil was divided into three applications with the following sources: potassium phosphate dibasic (K_2HPO_4), potassium phosphate monobasic (KH_2PO_4), ammonium nitrate (NH_4NO_3) and ammonium dihydrogen phosphate ($NH_4H_2PO_4$). In addition to those actions, ten applications of 100 mL of a nutrient solution of 10 mM calcium hydroxide ($Ca(OH)_2$) were carried out throughout the entire cycle of the plants. Selenium application was performed when the plants were in the V4 stage.

2.3. Sampling, harvesting, and grain yield

The V2 leaf was collected when the plants reached the flowering stage to quantify the hydrogen peroxide (H_2O_2), lipid peroxidation (MDA), as well as the activities of superoxide dismutase (SOD, EC:1.15.1.1), catalase (CAT, EC: 1.11.1.6), and ascorbate peroxidase (APX,

EC: 1.11.1.11). After collection, they were immediately conditioned in liquid nitrogen and stored at -80°C for biochemical analysis.

At the end of the cycle, the plants were harvested and separated into grain, shoot, and root. Sorghum grains were weighed to determine the grain yield in grams per plant. According to the Seed Analysis Rule (MAPA, 2009), grain moisture was determined, and grain yield was converted into dry mass by a correction of 13% moisture. The collected roots were washed to remove all soil residues.

All plant material (grains, shoots, and roots) were dried in a drying oven with forced air circulation and a temperature of ± 60°C until constant weight (after ± 72 hours). After drying, the material was ground in an electric grinder. The grain dry mass was used to quantify total carbohydrates. Selenium, macronutrients, and micronutrients were quantified both in grain and shoot dry mass. Selenium was also quantified in root dry mass.

2.3. Biochemical analyzes

2.3.1. Hydrogen peroxide (H_2O_2) and lipid peroxidation (MDA)

A mass of 0.4 g of leaves was macerated in liquid nitrogen and PVPP (polyvinylpolypyrrolidone), which was homogenized in 1.5 mL of 0.1 (w/v) trichloroacetic acid (TCA) and centrifuged at 12000 g for 15 minutes at 4°C. In the obtained extract, hydrogen peroxide (H_2O_2), was quantified as described in Velikova, Yordanov and Edreva (2000) and based on a standard curve with known H_2O_2 concentrations. Lipid peroxidation was determined as described by Buege and Aust (1978) in a spectrophotometer (Epoch-BioTek-Elisa) with readings in the absorbances of 535 and 600 nm, and the results were expressed in nanomoles of malondialdehyde (MDA) per gram of fresh mass (FM).

2.3.2. Extraction and Quantification of Antioxidant Enzymes

A leaf dry mass of 0.2 g was macerated in liquid N_2 with PVPP (polyvinylpolypyrrolidone) and homogenized with 1.5 mL of buffer solution (0.1 mol L^{-1} potassium phosphate - pH 7.8, 0.1 mmol L^{-1} EDTA - pH 7.0, and 0.01 mol L^{-1} ascorbic acid), as well as subsequently centrifuged at 13000 g for 10 minutes at 4°C and the supernatant was collected and stored at -20°C (Biemelt et al., 1998). The collected supernatant was used for the enzymatic analysis of superoxide dismutase (SOD) (Giannopolitis and Ries, 1977), catalase (CAT) (Havir and Mchale, 1987), and ascorbate peroxidase (APX) (Nakano and Asada, 1981) with readings performed in a spectrophotometer (Epoch BioTek-Elisa) with

560, 240, and 290 nm, respectively.

2.3.3. Total carbohydrates

For the quantification of carbohydrates, 0.2 g of the dry mass of the grains were homogenized with a buffer solution (0.1 mol L^{-1} potassium phosphate, pH 7.8) and incubated for 30 minutes in a water bath at 40°C, and later centrifuged at 16770 g for ten minutes (Zanandrea et al., 2009). An aliquot of the supernatant was removed for sucrose extraction, and a buffer solution (30% potassium hydroxide) was added to that aliquot. It was then incubated at 35°C for 15 minutes, as described by Van Handel (1968). After this process, for starch extraction, a buffer solution (enzyme amyloglucosidae and potassium acetate 200 mmol L^{-1}, pH4.8) was added to the pellet and incubated again at 40°C for two hours and then centrifuged at 16770 g for ten minutes. The contents of starch and sucrose were determined by the Antrona method (Yemm and Willis, 1954). The reducing sugars were determined according to the DNS (dinitrosalicylic acid) method (Miller, 1959)

2.5. Sample digestion procedure for elemental analyses

A dry mass of 0.5 g of each sample was separated for digestion following the 3051A method (USEPA, 2007). Each aliquot was digested with 5 mL of HNO_3 in Teflon® tubes (CEM Corporation, Matthews, NC, USA). The extract was left to stand overnight at room temperature, and the digestion was carried out the following morning. Next, the tubes were hermetically sealed and taken to a microwave (CEM brand, model Mars-5), with a temperature adjusted to 175°C and a controlled pressure of 0.76 MPa for 15 minutes. After digestion, the extracts were cooled to room temperature. The obtained extract was supplemented with an additional 5 mL of deionized water, and the extracts were transferred to bottles (30 mL), followed by storage at 5°C until analysis.

2.5.1. Determination of macronutrients and micronutrients

Determinations of the contents of selected macronutrients (S, P, K, Ca, Mg) and micronutrients (Fe, Cu, Zn, Mn) were performed by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES), using a Spectro equipment, model Blue (Germany), with background correction. The operating parameters and the sample introduction system were standardized according to the manufacturer's indication: plasma power of 1400 W, cooling gas flow rate of 12 L $minutes^{-1}$, auxiliary gas flow rate of 0.8 L $minutes^{-1,}$ and gas flow rate

the 0.85 L minutes^{-1} nebulizer. The gas used was argon with a purity ≥ of 99.99%. The determinations were carried out in a multi-element way; thus, the standard solutions were prepared using aliquots of standard stock solutions of 1000 mg L^{-1} of the elements under study. The solvent used in the procedure was also used for the dilutions of all solutions. The calibration curve for the proposed method had at least five standards of known concentration. The spectral line for each determining element is shown in supplementary material Table S2.

The N content was determined by sulfuric acid digestion and Kjeldahl distillation (Malavolta et al., 1997). A standard reference material - Tomato Leaves (SRM1573a) - and a blank sample were used for quality assurance/quality control (QA/QC) purposes. The average N recovery in this reference material (Tomato Leaves) was 118%.

2.5.2. Determination of Se

Selenium in the digested samples was analyzed by graphite furnace atomic absorption spectrometry (GFAAS) with Zeeman background correction and EDL lamp for Se, using a AAnalyst ™ 800 AAS equipment, Perkin Elmer. A standard solution containing 1 g kg^{-1} of Se (98% purity, Fluka, Buchs, Switzerland) was used to prepare the calibration curve for Se determination by GFAAS. A sample of standard reference material (White Clover - BCR 402, Institute of Reference Materials and Measurements, Geel, Belgium) for plant material was included in each batch of digestion for QA/QC purposes, along with a blank sample used to calculate the limits of detection and quantification. The average Se recovery in this reference material (White clover) was 98% (n = 7, [Se] = 6.57 mg kg^{-1}).

The limits of detection (LOD) and quantification (LOQ) were stipulated through ten readings of blank samples, following the same procedure adopted for plant material samples. The values were calculated with three and ten times the standard deviation of ten blank sample readings to determine LOD and LOQ, respectively (Khan et al., 2013). In the analysis of the materials under study, the LOD was 155 µg kg^{-1}, and the LOQ was 515 µg kg^{-1} of Se.

2.6. Nutritional efficiency of Se

Selenium content was related to the dry mass, and thus the Se accumulation was determined in each part of the plant and the entire plant for each treatment. To study the efficiency of Se use by sorghum plants, mathematical expressions of nutritional efficiency concepts proposed by several studies were applied, which estimated the following indices: Se absorption efficiency by roots, given in mg g^{-1} (Boldrin et al., 2012); Se recovery by the entire

plant shown in % (Boldrin et al., 2012); translocation efficiency of Se from the roots to the shoot + grains – Tr-Ef-Rt-ShGr (Li et al., 1991), and translocation efficiency of Se from the shoot to the grain - Tr-Ef-Sh-Gr, given in % (Ramkissoon et al., 2019).

Absorption efficiency = Se content in entire plant /root dry mass (Eq. A1)

Se recovery = (Se content in entire plant / Se applied) x 100 (Eq. A2)

Tr-Ef-Rt-ShGr = (Se content in shoot / Se content in entire plant) x 100 (Eq. A3)

Tr-Ef-Sh-Gr = (Se content in grains / Se content in shoot + grains) x 100 (Eq. A4)

2.7. Statistical analysis

The data were assessed for normality by the Shapiro-Wilk test, and for homogeneity of variance by the Bartlett test, followed by a subsequent statistical analysis of variance. The FDR test was used to compare the averages and to control false positives (reduction of type I error) (Benjamini and Hochberg, 1995). Ten treatments were considered to compare the factorial difference with the additional treatments (control and 480 µg Se dm^{-3} via sodium selenate). Without fixing one factor about the others, this was a more appropriate approach to indicate the most promising treatment. The simple linear relationship between the variables was carried out through a simple Pearson's correlation.

According to Cao et al. (1999), the data were standardized before the samples are agglomerated because the variables are measured in very different units. A principal component analysis (PCA) was performed to report the variation in doses for each Se source. This analysis allowed the characterization of the variables that discriminated the most against the structural characteristics in each treatment. Thus, the initial set of variables started to be described by two new orthogonal latent variables, making it possible to locate them in two-dimensional figures (Hair Junior et al., 2009). The statistical analyses and the graphs were carried out with the software R (R Core Team, 2020).

3. Results

The analysis of variance (Supplementary material Table S3) revealed that the response of sorghum plants was dependent on the interaction between Se doses and sources (D x S) for the variables: Se content in grains, shoot, and root; Se absorption efficiency; Se recovery; translocation efficiency of Se from the root to the shoot + grains and shoot to the grain; S, K, Cu, Fe, Mn, Zn contents in the grain; S, P, Fe content in the shoot; MDA; SOD; APX; reducing sugars and starch.

Although the interaction effect (D x S) was not significant, sorghum plants showed responses dependent only on the Se source for the variables: K, Ca, and Zn content in the shoot; CAT; and sucrose. In contrast, H_2O_2 and the Mn content in the shoot was mainly influenced by the Se dose. The additional treatment (480 µg dm^{-3} of Se through sodium selenate) had a significant effect ($p \leq 0.05$) on N content in the grain. However, the treatments applied did not significantly differ for the variables: dry mass root; P and Mg content in the grain; Mg, N, and Cu content in the shoot.

3.1. Selenium content

Selenium content in the grain (Fig. 1A) and stem (Fig. 1B) increased with increasing Se doses, so that the additional treatment had the highest Se content. Selenium content in the control treatment could not be detected because it was below the detection (LOD) and quantification (LOQ) limit. Selenium content in grains with the additional treatment was 99.2, 98.6, 97.5 and 89.4% higher than SeA, SeB, SeC and SeD with the 60 µg Se dm^{-3}, respectively. The additional treatment was also 96.2, 93.0, 93.1 and 37.6% higher than via SeA, SeB, SeC and SeD with 240 µg Se dm^{-3}, respectively. Selenium content in the shoots with the SeD application at the Se doses of 60 and 240 µg dm^{-3} was 86.8 and 40.3% lesser than the additional treatment, respectively.

Selenium content in the root (Fig. 1C) with the application of SeA at the Se doses of 60 and 240 µg dm^{-3} was reduced by 13.0 and 12.5%, respectively when compared with the additional treatment. The lowest Se content in the root occurred with 60 µg Se dm^{-3} applied as SeD. With the Se dose of 60 µg dm^{-3}, there was a higher percentage of Se accumulation in the roots in all sources of Se applied (Fig. 1H).

3.2. Nutritional efficiency of Se

The absorption efficiency of Se (Fig. 1D) concerning the total Se accumulated in the plant per gram of root produced was higher with the Se dose of 240 µg dm^{-3} compared with 60 µg Se dm^{-3} supplied with SeD. The greatest absorption efficiency of Se occurred with an additional treatment containing the highest applied Se dose. The translocation efficiency of Se from root to shoot + grain (Fig. 1E) was not significantly different between the additional treatment and 240 µg Se dm^{-3} through SeD, which had 95.9 and 96.2% respectively of translocation efficiency of Se.

The SeA, SeB, SeC sources with the Se dose of 240 µg dm^{-3} had a higher percentage

of translocation than with the Se dose of 60 µg dm^{-3}. However, the highest translocation efficiency of Se from shoot to grain (Fig. 1F) was 64.8 and 63.8%, with 60 µg Se dm^{-3} through SeA and SeB, respectively. Selenium sources, SeB at a Se dose of 60 µg dm^{-3} and SeC at Se doses of 60 and 240 µg dm^{-3} showed mobilities similar to SeD at the Se doses in this study, with an average between 35.1 and 46.3% translocation efficiency of Se from shoot to grain. Selenium source SeD provided better Se recovery (Fig. 1G) of the applied Se when compared with the other Se sources. However, this was not observed with the 480 µg Se dm^{-3}, where there was a 15.5% reduction in Se recovery compared with 240 µg Se dm^{-3} via SeD.

3.3. Antioxidant metabolism

Lipid peroxidation quantified by MDA content (Fig. 2A) was increased by the Se application, except when applying 60 µg Se dm^{-3} through SeC, which did not differ from the control treatment (without Se application). However, SeC with 240 µg Se dm^{-3} had the highest MDA content with increments of 44.3 and 16.9% compared with the control and the additional treatment, respectively. Selenium sources did not significantly influence the hydrogen peroxide (H_2O_2) content (Fig. 2B). However, Se doses of 60, 240, and 480 µg dm^{-3} (as SeD) increased by 23.6, 29.4, and 36.7%, respectively, compared with the control treatment.

The catalase activity (CAT) was influenced only by Se sources (Fig. 2C). SeC, SeD, and the additional treatment increased the activity of this enzyme by 35.8, 33.1, and 43.8%, respectively, compared with the control treatment. The activity of superoxide dismutase (SOD) (Fig. 2D) was noteworthy with 240 µg Se dm^{-3} via SeA, which increased the activity of these enzymes by 27.2 and 32.2% compared with the control and the additional treatment. The highest ascorbate peroxidase (APX) activities (Fig. 2E) occurred with 240 µg Se dm^{-3} applied as SeB and 60 µg Se dm^{-3} as SeC. These treatments generated increments of 44.8 and 36.2% in APX compared with the control treatment, respectively. Moreover, they also increase by 36.8 and 26.9%, respectively, the activity of this enzyme when contrasted with the additional treatment.

3.4. Carbohydrate, nitrogen, and grain yield

Selenium application significantly influenced the carbohydrate content in the grain. Selenium dose of 60 µg dm^{-3} via SeA increased by 41.7 and 23.0% the reducing sugars when compared with the control and the additional treatment (Fig. 3A). The reducing sugars were

also higher with 60 µg dm^{-3} Se than with 240 µg Se dm^{-3} via SeA and SeB. However, with the SeC and SeD sources, the reducing sugars did not differ significantly between the Se doses of 60 and 240 µg Se dm^{-3}. On the other hand, sucrose (Fig. 3B) showed no significant difference between doses and was influenced only by the applied Se sources. The sources SeA, SeC, SeD and the additional treatment had higher sucrose contents than the SeB and the control treatment. Starch (Fig. 3C) in the control treatment was 20.3 and 10.7% lesser than with Se applied at doses of 240 and 60 µg dm^{-3} via SeD and SeA, respectively.

The N content in the grain had a significant difference between the factorial and the additional treatment by the F test ($p \leq 0.05$), but this difference was not detected with the mean test (Fig. 3D). Grain yield did not show significant differences among the applied Se sources. However, when considering the doses of Se applied, the main difference occurred between the Se doses of 60 and 480 µg dm^{-3}, which differed by 11.8% (Fig. 3E).

3.5. Macronutrients

The S content in the grain (Fig. 4A) with 60 and 240 µg Se dm^{-3} applied through SeB and SeA was higher by 9.3 and 9.9%, respectively, than with 60 µg Se dm^{-3} via SeC. The additional treatment has shown reductions of 10.0 and 10.6% compared with 60 and 240 µg Se dm^{-3} applied as SeB and SeA, respectively. The application of SeD at a Se dose 240 µg Se dm^{-3} reduced by 7.1% the S content in the grain compared with a dose of 60 µg Se dm^{-3}. The S content in the shoot (Fig. 4B) with 60 µg dm^{-3} Se was 11.6 and 15.3% lower than 240 µg dm^{-3} Se as SeC and the additional treatment, respectively. The P content in the shoot (Fig. 4C) with 60 µg dm^{-3} Se was 14.5% higher than with 240 µg dm^{-3} Se as SeC, whereas the opposite effect was observed with 60 µg dm^{-3} Se via SeD, which was 15.3% smaller than 240 µg dm^{-3} Se. The shoot P content with 240 µg Se dm^{-3} via SeD increased by 30.1% compared with the additional treatment and did not differ from the control treatment.

The K content in grains (Fig. 4D) with the application of SeB at a Se dose of 60 µg dm^{-3} was 13.1, 25.5, and 39.1% higher than when 240 µg Se dm^{-3} was applied through SeB, the control, and the additional treatment, respectively. The K contents at the shoot with SeA, SeB, and SeC were correspondingly 14.7, 13.1, and 9.6% higher than the control treatment. These Se sources also increased by 16.0, 14.4, and 11.1%, respectively, the shoot K contents when contrasted with the additional treatment. Moreover, the shoot K content (Fig. 4E and 4F) for 60 µg Se dm^{-3} was 12.1 and 13.4% higher than the control and the additional treatments, whereas with 240 µg Se dm^{-3}, there were increments of 8.4 and 7.8% when

compared with the control and the additional treatment, respectively. Finally, the Ca content in the shoot (Fig. 4G and 4H) with SeA and the additional treatment were 32.8 and 26.9% higher than the control treatment. Furthermore, with Se application at the doses of 60, 240, and 480 µg dm^{-3}, there were increases in Ca content in the shoot by 26.1, 24.3, and 26.9%, respectively, when compared with the control treatment.

3.6. Micronutrients

The Fe content in the grain (Fig. 5A) with 60 and 240 µg Se dm^{-3} applied through SeB and SeA was 28.0 and 32.8% higher than with the control treatment, respectively. These treatments also increased Fe content in the grain by 23.7 and 28.3%, respectively, when compared with the additional treatment. Selenium application of 240 µg Se dm^{-3} through SeA and SeD provided higher Fe contents in the grain - i.e., 32.4 and 16.7%, respectively - than when Se was applied at the dose of 60 µg dm^{-3} via these Se sources. Conversely, the application of SeB with 240 µg Se dm^{-3} had a reduction of 18.9% compared with 60 µg Se dm^{-3}. Overall, the Se supply resulted in reduced Fe content in the shoot (Fig. 5B), except when applied at the dose of 240 µg dm^{-3} Se as SeC, which was 18.6% higher than 60 µg dm^{-3} Se in this same source. Indeed, the Fe content in the shoot with 240 µg Se dm^{-3} applied through SeA and SeB was 10.5 and 9.3% respectively, less than 60 µg Se dm^{-3} with these same sources. The additional treatment also showed a reduction of 38.8% compared with the control treatment.

The highest Zn content in the grain (Fig. 5C) occurred with 60 µg Se dm^{-3} as SeB, which was 26.6% higher than the control treatment. The Zn content in the grain with SeA, SeC, and SeD at 240 µg Se dm^{-3} resulted in increments of 15.7, 6.1, and 10.4%, respectively, when compared with the dose of 60 µg Se dm^{-3}. Generally, the Se sources influenced shoot Zn content (Fig. 5D) regardless of the Se dose applied, with a difference of 8.2% between SeA and SeD. The Mn content in the shoot (Fig. 5E) was influenced only by the Se doses applied. Selenium doses of 60 and 240 µg dm^{-3} provided 20.5 and 17.8% increments, respectively, when compared with the control treatment.

Last of all, the application of 60 and 240 µg Se dm^{-3} via by SeB provided the highest Cu content in the grain (Fig. 5F), with increments of 30.9 and 38.5% compared with the control treatment, respectively. Selenium dose of 60 µg dm^{-3} resulted in increments 24.8% compared with 240 µg Se dm^{-3} applied as SeA. However, 60 µg Se dm^{-3} via SeD resulted in a reduction of 15.5% compared with 240 µg Se dm^{-3} with this same Se source.

3.7. Principal Component Analysis and Pearson's correlation

The principal component analysis (Fig. 6) provided a better understanding of the overall effect of Se fertilization on the variables evaluated in this study. The first two principal components accounted for 54.4% of the total variation. The first component (horizontal axis) represented most of the total variation, and treatments were separated in terms of Se doses and sources applied. The grouping on the right shows the variable responses influenced by the additional treatment and by 240 µg Se dm^{-3} applied as SeD, which indicated increased Se content, Se accumulation in grains, Se uptake efficiency by roots, grain yield, and SC-Sh. The grouping on the left shows the variables that received Se via SeA and SeB at the doses of 60 and 240 µg dm^{-3}, which indicated an increase in the content of Cu, K, Mn, and S in the grains; Mn, Zn, and K content in the shoot; SOD and APX.

The Pearson's correlations (Fig. 7) for the evaluated variables showed a significant positive correlation ($p \leq 0.05$) between CAT and Se content in the shoot ($r = 0.58$) as well as in the grain ($r = 0.57$), absorption efficiency ($r = 0.56$), and Se recovery ($r = 0.55$). The significant positive correlation indicated that with the increase in Se content in the grain there was also an increase in the absorption efficiency ($r = 0.99$), Se recovery ($r = 0.77$), CAT ($r = 0.67$), translocation efficiency from root to shoot + grain ($r = 0.61$), sucrose ($r = 0.29$), grain yield ($r = 0.40$), S content in the shoot ($r = 0.41$), starch ($r = 0.26$), Se content in the root ($r = 0.46$) , MDA ($r = 0.25$), H_2O_2 ($r = 0.36$) and Cu content in the shoot ($r = 0.09$). Among the nutrients present in the grains, Mg and P had the highest magnitude of significant correlation ($r = 0.80$), but they were not significant in relation to Se. Significant negative correlations were observed between Se content in grains and N ($r = -0.21$), Zn ($r = -0.15$), Fe ($r = -0.41$), Mn ($r = -0.29$), K ($r = -0.50$) content in shoot; Fe ($r = -0.05$), Mn ($r = -0.12$), S ($r = -0.18$) N ($r = -0.37$), Cu ($r = -0.37$), K ($r = -0.68$) content in the grain; and SOD ($r = -0.46$).

4. Discussion

Selenium fertilization via the source “selenate” (i.e., SeD) had the highest Se levels in the sorghum plant parts (Fig. 1A, 1B and 1C). This is due to the higher absorption efficiency of Se of the root, Se recovery, and translocation efficiency of Se from root to shoot +grains (Fig. 1D, 1E, 1F and 1G). Boldrin et al. (2012) observed a similar result for the application of selenate in rice plants. The metabolic phases covered in Se absorption, mobilization, and assimilation are deeply linked with S because selenate can utilize the high-affinity sulfate transporters that contribute to the sulfate absorption and transport through plant tissues (Sors

et al., 2005).

With Se application via soil, this element is exposed to several factors that affect its mobility and solubility. Consequently, the efficiency of Se absorption by plants is also affected due to the alteration of Se mobility and solubility. The factors influencing Se in the soil are pH, sorption, and desorption reactions, redox potential, organic/inorganic compounds, and dissolution processes in soils and sediments (Schiavon et al., 2020). When Se in soil prevails in anionic form, it can be absorbed by aluminum or iron oxides/hydroxides or anionic clays by electrostatic interactions (Winkel et al., 2015). Selenium in the form of selenide in the soil can bind to organic matter (Gustafsson and Johnsson, 1994), which influences Se retention and bioavailability, the formation of organominerals that protect Se adsorbed on (oxy)hydroxides, and the reduction of Se oxyanions to Se elemental (Kaush and Pallud, 2013; Tolu et al., 2014). Although it was considered that higher plants do not require this element, the experience of Finnish soils with low Se content provides evidence that the supplementation of commercial fertilizers with sodium selenate had a positive impact on Se transfer from the soil to plants, animals, and humans (Hartikainen, 2005).

Selenium provides changes in carbohydrate as a function of concentration, source of Se, and phenological stage. In the present study, Se doses and sources caused contradictory effects on carbohydrate (reducing sugars, sucrose, and starch) (Fig. 3A, 3B and 3C). In some cases, it provided increases, and in others, decreases. Similar effects were observed by Kaur et al. (2018) in wheat grains with the application of selenate and selenite. Disaccharides, such as sucrose, are soluble sugars with an antioxidant function (Keunen et al., 2013). These carbohydrates increase stress levels because they are directly associated with the preservation of cellular homeostasis by the balance generated in reactive oxygen species (ROS), mainly produced by hydroxyl radicals (Coueé et al., 2006).

Studies carried out to identify biochemical alterations in plants correlated with Se insertion are essential for the biofortification of a crop. Although no symptoms of phytotoxicity occurred with the application of different Se doses and sources, the increase in the content of MDA and H_2O_2 with Se supply (Fig. 2A and 2B) indicates that there may have been a stress condition since the hydrogen peroxide (H_2O_2) acts as an active oxide stress signal (Blokhina and Fagerstedt, 2010) due to its ability to cross cell membranes in a longer half-life. Furthermore, the increased activity of antioxidant enzymes or more marked synthesis possibly indicates a signal of the need for greater ROS's control.

Both unstressed and stressed cells produce ROS. When there are no stressing

conditions, O_2 formation and removal are in balance. However, under stressful conditions, ROS formation can increase to the point of overloading the defense system (Alscher et al., 2002). According to Gill and Tuteja (2010), when generated in excess, ROS can cause additional cell damage; however, when in low concentrations, they act as molecular signalers, which trigger positive signaling cascades to the effect of compensatory metabolism in plants.

The superoxide dismutase (SOD) (Fig. 2D) is the first line of defense against ROS (Alscher et al., 2002). The SOD activity may have been favored by a higher ROS production from superoxide radicals, which provided higher H_2O_2 concentrations. Others authors have reported increased SOD activity with Se supply when plants were under stress conditions, e.g., potatoes with application of selenate and selenite (Seppänen et al., 2003) and due to water deficit, e.g., rice with application of selenate (Andrade et al., 2018). The increase in CAT (Fig. 2C) enzyme activity with Se through SeC and SeD may have helped maintain the MDA and H_2O_2 content, preventing these contents from being more prominent and likely to cause damage, increasing the control capacity of the enzymatic antioxidant system. Feng et al. (2016) observed an increase in the activity of APX and CAT with Se supply, in rice (with selenite application), indicating the potentiation of the antioxidant system, as in the present study, promoted by the presence of Se in ideal concentrations.

CAT and APX (Fig. 2C and 2E) can remove H_2O_2 through different mechanisms, leading to water production. The specificity between enzymes and H_2O_2 reflects their different affinities, where APX has a high affinity for H_2O_2, while CAT has a low affinity. Therefore, APX will be responsible for fine-tuning these ROS, while CAT will remove excess ROS under pressure. Therefore, CAT and APX are essential in plant cells, and special attention was given to studies that aim to understand their interactions under different types of stress (Foyer and Noctor, 2003).

Recent studies have shown that Se in low concentrations can protect plants from various types of abiotic stresses (Abbas 2012; Andrade et al., 2018; Djanaguiraman et al., 2010). Selenium can increase the tolerance of plants exposed to high temperatures (Djanaguiraman et al., 2010), low temperatures (Abbas, 2012), and water stress (Andrade et al., 2018; Ravello et al., 2021). According to Djanaguiraman et al. (2010), Se can play a protective role during high-temperature stress, increasing the antioxidant defense system in sorghum supplied with Se, as selenate. The protective function performed by Se in protecting plants from cold stress is likely due to the reduction of oxygen radicals, osmotic regulation by synthesis of an osmoregulatory compound, and increased biosynthesis of enzymatic and non-

enzymatic antioxidants in sorghum with selenate application (Abbas, 2012). In rice plants with selenate application, Andrade et al. (2018) observed that the application of Se improved the CO_2 assimilation rate, the transpiration rate, the instantaneous carboxylation efficiency, the estimation of the water use efficiency, and the physiological capacity to withstand the water deficit. Similar effects have been reported also, for legumes such as beans (Ravello et al., 2021).

The reduction in the N content in the grain (Fig. 3D) with the highest Se dose applied in this study is probably due to a dilution effect since, in this treatment, there was the highest grain yield (Fig. 3E). The above-mentioned effect may occur since the demand for N is high to maintain the typical plant metabolism (Miller and Cramer, 2004), not only for protein synthesis but also for synthesizing other macromolecules. Application of N enhances S metabolism, increases S assimilation, increasing O-acetylserine, a key regulator of S metabolism in cysteine synthesis, and then increases cysteine and protein synthesis (Kim et al., 1999). Since Se and S use the same metabolic pathway in plants, one could expect that the application of N can also promote the uptake of Se by the plant, and consequently Se can be metabolized into selenoproteins (Zhou et al., 2020).

Supplying Se to plants can alter the absorption and accumulation of minerals essential for plant metabolism (Djanaguiraman et al., 2010). Freeman et al. (2010), observed in *Stanleya pinnata* treated with selenate that the molecular mechanism controlling Se accumulation in plants had greater expression of genes involved in the assimilation of sulfur. When evaluating the effect of Se application on the mineral content of sorghum plants, a contradictory response was observed. Reducing the S content in the grain (Fig. 4A) may indicate competitive inhibition between Se and S since selenato and S are absorbed by the same metabolic route (Sors et al., 2005), indicating an antagonism due to a high Se dose applied.

Some treatments did not have significant increases or decreases with Se fertilization, concerning the S content (Fig. 4A and 4B). Similar results were obtained by Lara et al. (2019) who did not observe the influence of increasing Se doses on the S content in wheat grains and shoots when the plant was treated with selenate. However, Boldrin et al. (2012) and Boldrin et al. (2013) observed an increase in the S content in rice grains and leaves, respectively, when plants were supplied with Se as sodium selenate.

The P content in the shoot of sorghum (Fig. 4C) was reduced with the increase in Se doses applied through SeC, a fact that is the opposite when SeD (selenate) was used instead,

demonstrating a possible competitive inhibition of SeC in the P absorption, resulting in a lower P content in the plants, as occurs when selenite is applied. Competitive inhibition also occurs between selenite and P in several plants. An increase in selenite or phosphate concentrations in the culture solution generates changes in the levels of Se and P (Hopper and Parker, 1999) in perennial ryegrass (*Lolium perenne* L. cv. Evening Shade) and strawberry clover (*Trifolium fragiferrum* L. cv. O'Conner).

The increments in K content in the grain (Fig. 4D) with SeA and SeB, and the reduction of this element as a function of the increase in Se doses via SeD (selenate) probably occurred due to the dilution effect of this nutrient as a function of the increment in the grain yield with the application of SeD. The K content in the shoot (Fig. 4E and 4F) showed similar behavior to the K content in the grain. Different effects of Se applied as sodium selenate (e.g., SeD in this study) on K content in shoots of maize plants were reported by Hawrylak-Nowak (2008). This author observed that in the presence of 25 µmol Se dm^{-3}, the K content increased, while with 100 µmol Se dm^{-3} in the nutrient solution, the content of this element decreased when compared with the control treatment.

Selenium application did not significantly change the Mg content in the shoot and the grains with none of the Se doses and sources. In studies with maize, there was no effect on Mg contents when using Se through selenate (Hawrylak-Nowak, 2008) and selenite (Pazurkiewicz-Kocot et al., 2008). Studies with the application of Se organic sources in plants are still scarce. The Ca content in the shoot (Fig. 4G and 4H) showed an increasing trend in conjunction with the increase in the Se dose applied, with significant increases with all Se sources when contrasted with the control treatment. However, there was no significant interaction between Se doses and sources. Hawrylak-Nowak (2008) also observed increases in the Ca content in the shoot of Se-treated in maize plants when compared with the control (plants without Se).

In general, it was found that Se supply changed the Fe, Zn, Cu, and Mn contents in sorghum plants (Fig. 5A, 5B, 5C, 5D, 5E and 5F). In fact, the presence of Se in plants can change the ionic permeability coefficient in the plasma membrane, thus altering the transport and accumulation of micronutrients in plant cells. Furthermore, these can be the first signal of Se effects on plants (Pazurkiewicz-Kocot et al., 2008). As Cu, Fe, Zn, and Mn are cofactors of SOD (Alscher et al., 2002), the changes observed in these elements may be related to the activity of this enzyme. Possible synergisms or antagonisms between Se and micronutrients may be related to the Se source applied.

Finally, the increase in Fe, Zn, Mn, and Cu contents in the grains observed with the 60 µg Se dm^{-3} applied through SeB, and with 240 µg Se dm^{-3} via SeA may indicate a possible synergistic effect of these sources with those elements. Moreover, selenate showed a synergistic effect due to increases in the Cu, Zn, and Mn contents in rice grain (Boldrin et al., 2013).

5. Conclusion

The efficiency of different doses and sources of selenium in the biofortification of sorghum with Se, as well as their effect in the antioxidant system and the contents of carbohydrates, were evaluated in this study in response to soil Se application. Considering the parameters evaluated, the Se sources SeB (methyl hydroxy-selenide) and SeC (hydroxy-selenide) at a dose of 240 µg Se dm^{-3} and SeD (sodium selenate) at a dose of 60 µg Se dm^{-3} are considered promising for the biofortification of sorghum. Selenium application via sodium selenate - SeD - was responsible for the greater absorption efficiency of the roots, better percentage of Se recovery, and translocation efficiency of Se from root to shoot + grains implying the highest Se content and accumulation observed in these parts of the plant. Selenium application promoted a change in the antioxidant metabolism to favor the control of excess ROS's by beneficially increasing the enzymatic activity of the antioxidant enzymes and the carbohydrate content in the grains. In summary, sorghum plants respond positively to the biofortification approach with Se.

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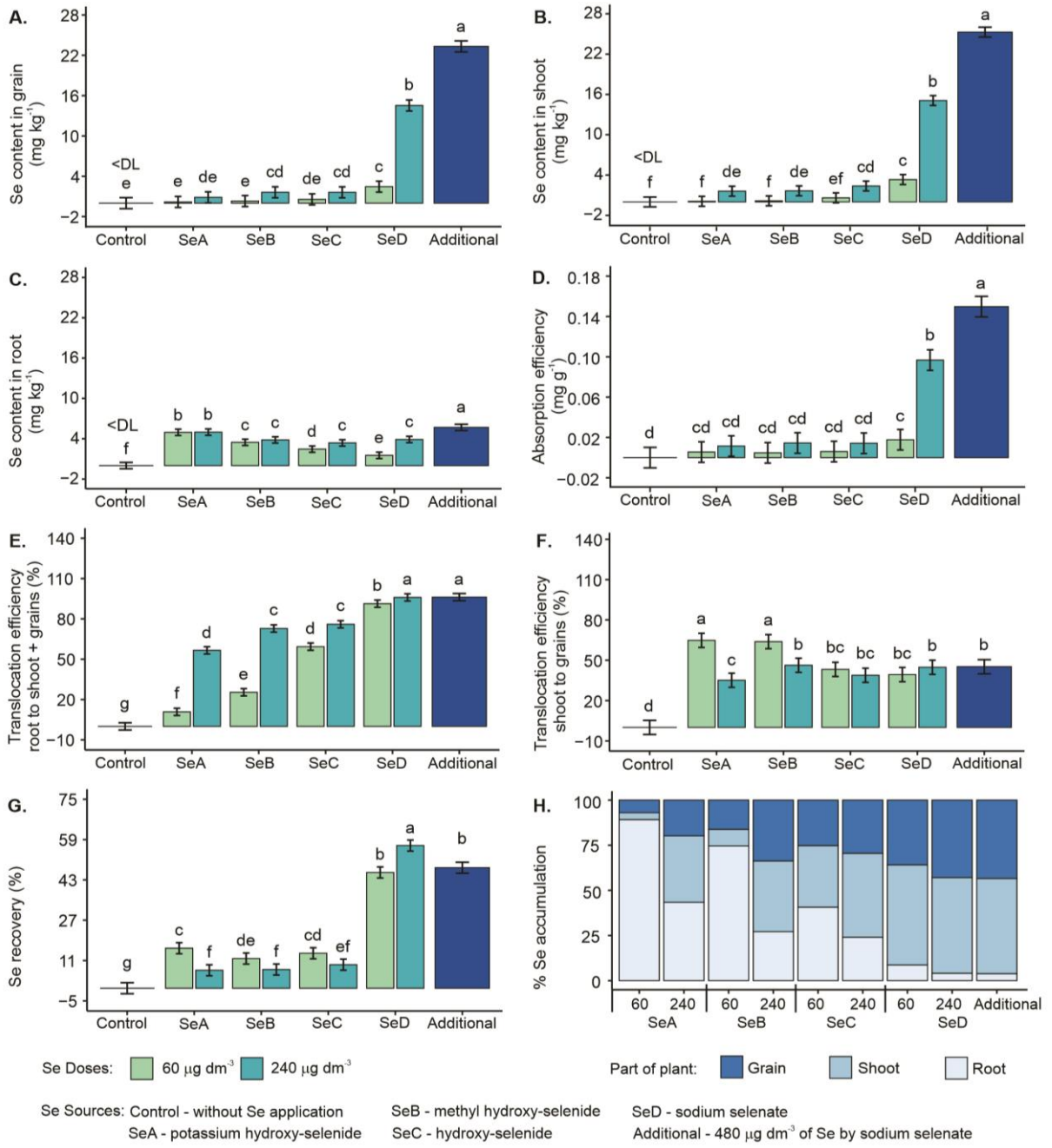

Fig. 1. Effect of selenium application via soil on the Se content in grain (A), Se content in shoot (B), Se content in root (C), absorption efficiency (D), translocation efficiency root to shoot +grain (E), translocation efficiency shoot to grain (F), Se recovery (G), and % Se accumulation in sorghum plants. Different letters indicate significant differences between treatments at a probability level of 5% ($p \leq 0.05$) by the FDR test. The bars show means, and the vertical error bars refer to the standard errors (n=4).

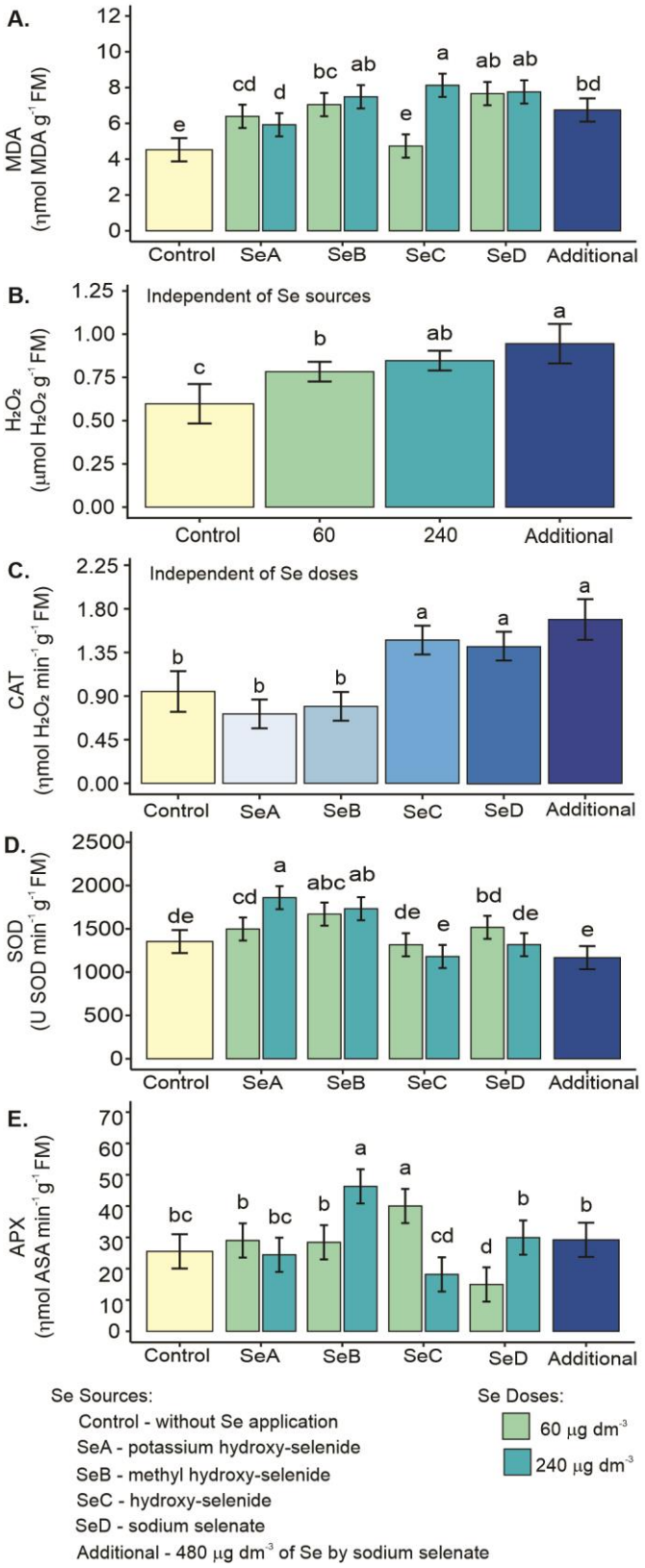

Fig. 2. Effect of selenium application via soil on the enzymatic activities of lipid peroxidation by the content of MDA (A); hydrogen peroxide - H_2O_2 (B); catalase - CAT (C); superoxide dismutase - SOD (D); ascorbate peroxidase - APX (E). Different letters indicate significant differences between treatments at a probability level of 5% ($p \leq 0.05$) by the FDR test. The bars show means, and the vertical error bars refer to the standard errors (n=4).

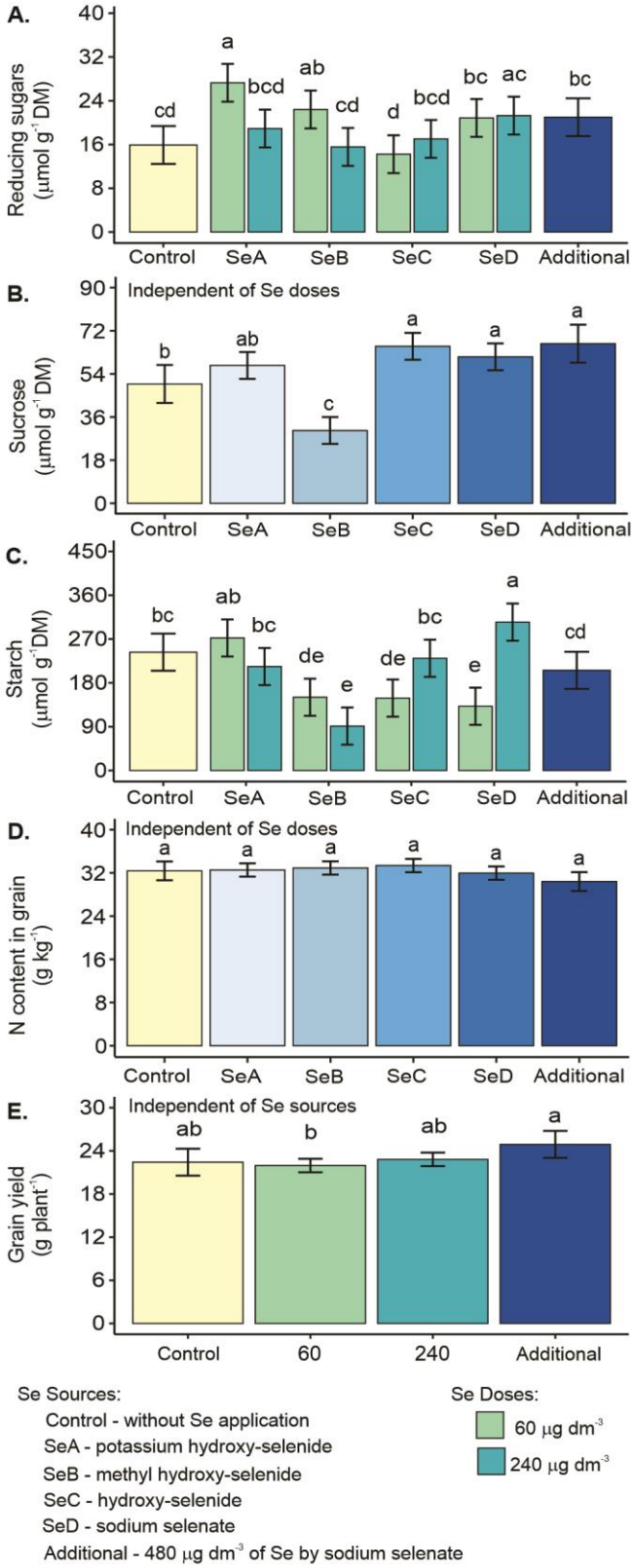

Fig. 3. Effect of selenium application via soil on the reducing sugars (A), sucrose (B), starch (C), N content in grain, and grain yield (E) in the grain sorghum. Different letters indicate significant differences between treatments at a probability level of 5% ($p \leq 0.05$) by the FDR test. The bars show means, and the vertical error bars refer to the standard errors (n=4).

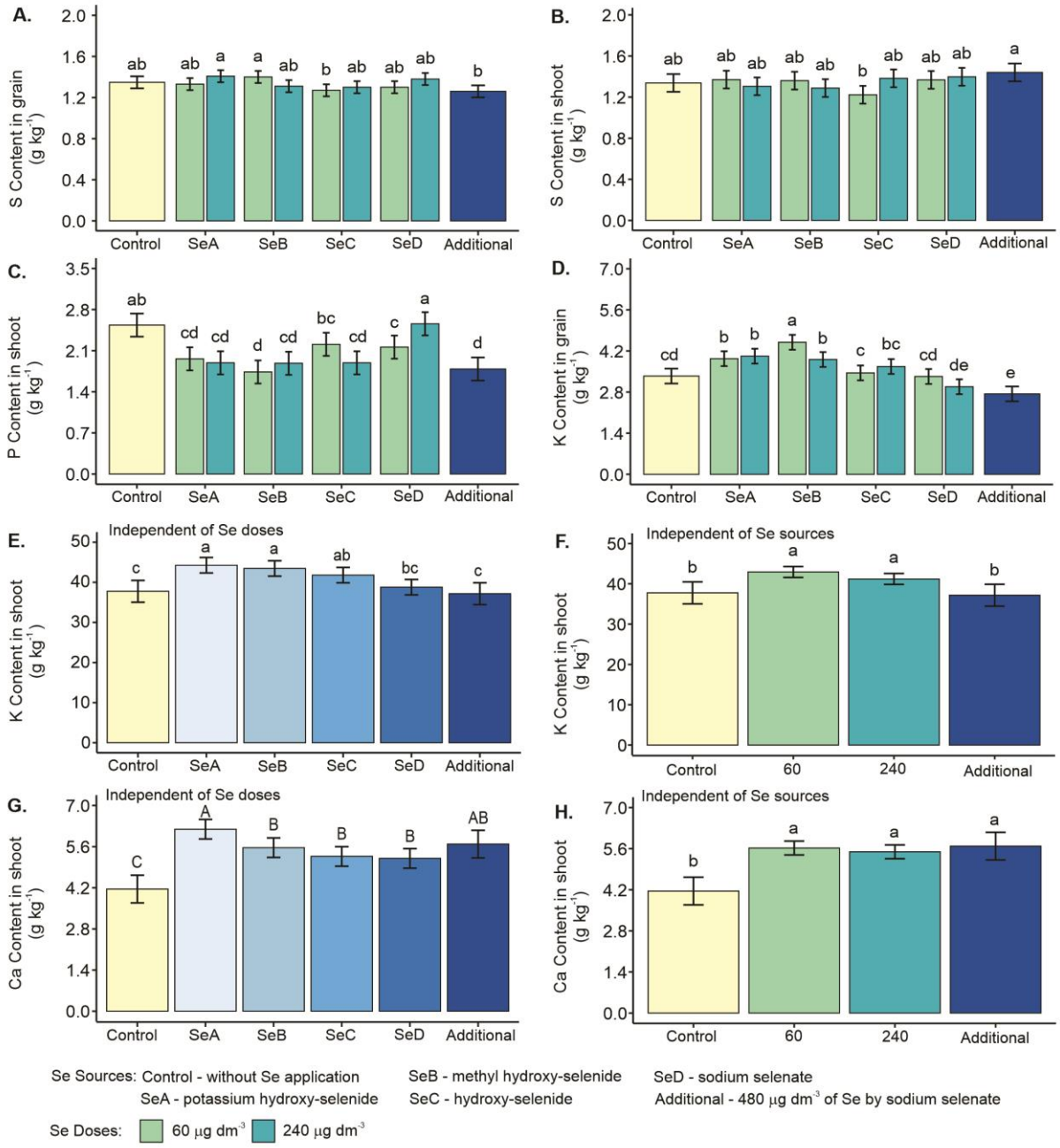

Fig. 4. Effect of Se application via soil on the S content in grain (A), S content in shoot (B), P content in shoot (C), K content in grain (D), K content in shoot (E) and (F), Ca content in shoot (G) and (H) in the sorghum plants. Different letters indicate significant differences between treatments at a probability level of 5% ($p \leq 0.05$) by the FDR test. The bars show means, and the vertical error bars refer to the standard errors (n=4).

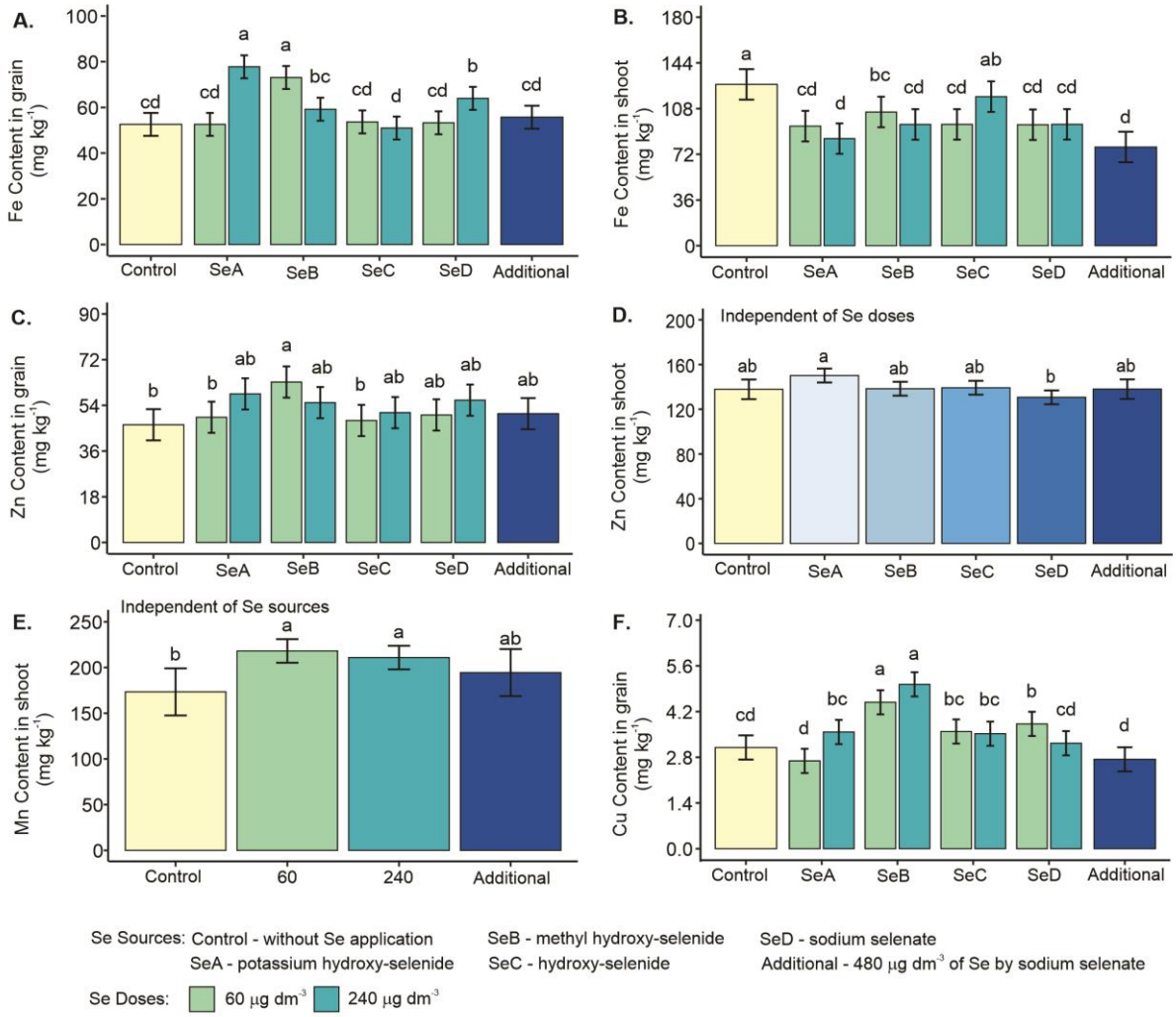

Fig. 5. Effect of Se application via soil on Fe content in grain (A), Fe content in shoot (B), Zn content in grain (C), Zn content in shoot (D), Mn content in shoot (E) and Cu content in grain (F) in the sorghum plants. Different letters indicate significant differences between treatments at a probability level of 5% ($p \leq 0.05$) by the FDR test. The bars show means, and the vertical error bars refer to the standard errors (n=4).

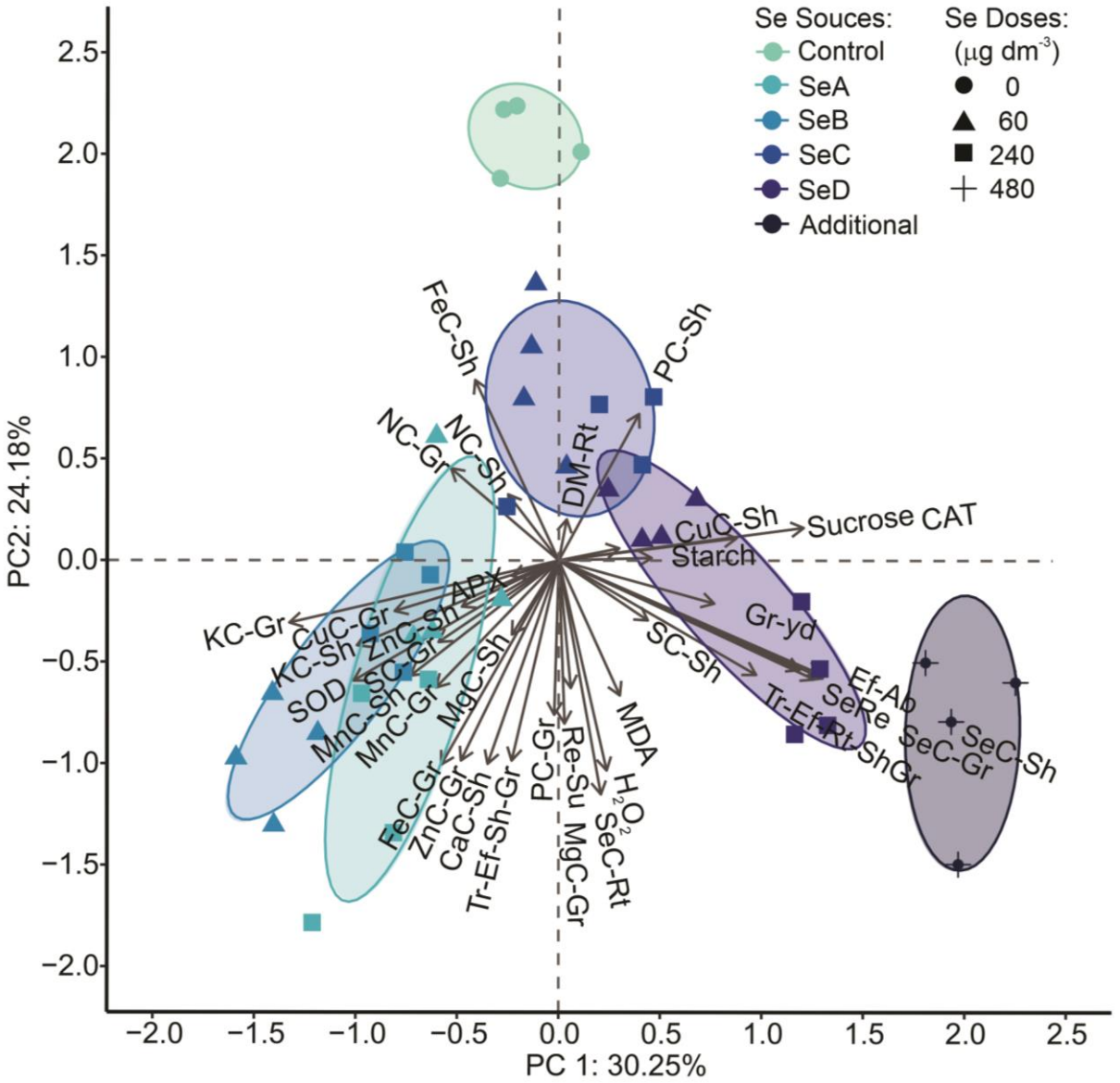

Fig. 6. Principal component analysis. Abbreviations: Se content in the grain (SeC-Gr), Se content in the shoot (SeC-Sh), Se content in the root (SeC-Rt), absorption efficiency (Ef-Ab), Se recovery (Se-Re), translocation efficiency root to shoot+grain (Tr-Ef-Rt-ShGr), translocation efficiency shoot to grains (Tr-Ef-Sh-Gr), lipid peroxidation (MDA), hydrogen peroxide (H_2O_2), catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX), reducing sugar (Re-Su), sucrose, starch, grain yield (Gr-yd), N (NC-Gr), S (SC-Gr), P (PC-Gr), K (KC-Gr), Mg (MgC-Gr), Fe (FeC-Gr), Zn (ZnC-Gr), Mn (MnC-Gr), Cu (CuC-Gr) content in the grain; N (NC-Sh), S (SC-Sh), P (PC-Sh), K (KC-Sh), Mg (MgC-Sh), Ca (CaC-Sh); Fe (FeC-Sh); Zn (ZnC-Sh), Mn (MnC-Sh) and Cu (CuC-Sh) content in the shoot.

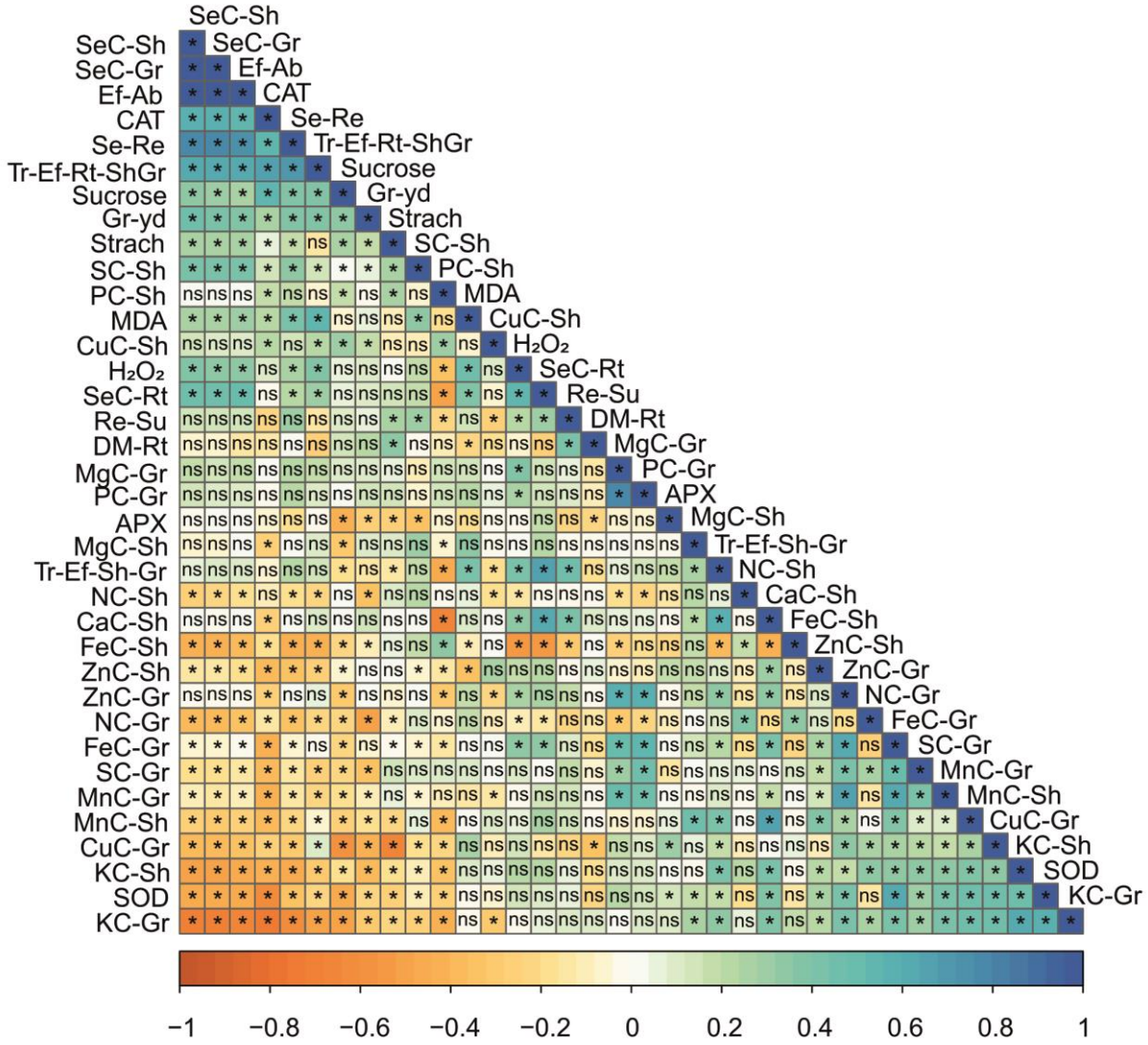

Fig. 7. Heatmap showing the Pearson's correlation the significance of relationship is identified with * significant by F-test at $p \leq 0.05$ and *ns* is not significant. Abbreviations: Se content in the grain (SeC-Gr), Se content in the shoot (SeC-Sh), Se content in the root (SeC-Rt), absorption efficiency (Ef-Ab), Se recovery (Se-Re), translocation efficiency root to shoot+grain (Tr-Ef-Rt-ShGr), translocation efficiency shoot to grains (Tr-Ef-Sh-Gr), lipid peroxidation (MDA), hydrogen peroxide (H_2O_2), catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX), reducing sugar (Re-Su), sucrose, starch, grain yield (Gr-yd), N (NC-Gr), S (SC-Gr), P (PC-Gr), K (KC-Gr), Mg (MgC-Gr), Fe (FeC-Gr), Zn (ZnC-Gr), Mn (MnC-Gr), Cu (CuC-Gr) content in the grain; N (NC-Sh), S (SC-Sh), P (PC-Sh), K (KC-Sh), Mg (MgC-Sh), Ca (CaC-Sh); Fe (FeC-Sh); Zn (ZnC-Sh), Mn (MnC-Sh) and Cu (CuC-Sh) content in the shoot.

Appendix A. Supplementary material

Table S1. Chemical and mineralogical characterization and particle size distribution of soil dystrophic Red-Yellow Latosol (Typic Haplustox) used in the greenhouse experiment before the fertilization.

Properties	Values	Units	Method/ Extractant	Properties	Values	Units	Method/ Extractant
Se[1]	0.064	mg kg^{-1}	USEPA 3051A	pH	5.1		Water 1:25
P	0.71	mg dm^{-3}	Mehlich-1	SOM	6.8	g kg^{-1}	$Na_2Cr_2O_7$ 4N + H_2SO_4 10N
K	23.46	mg dm^{-3}	Mehlich-1	Sand	730	g kg^{-1}	Hydrometer
Ca	0.19	$cmol_c$ dm^{-3}	KCl (1 mol L^{-1})	Silt	50	g kg^{-1}	Hydrometer
Mg	0.10	$cmol_c$ dm^{-3}	KCl (1 mol L^{-1})	Clay	220	g kg^{-1}	Hydrometer
S	4.23	mg dm^{-3}	Monocalcium phosphate in acetic acid	Al_2O_3	14.8	%	Acid digestion
B	0.07	mg dm^{-3}	Hot water	SiO_2	39.7	%	Acid digestion
Cu	0.10	mg dm^{-3}	Mehlich-1	P_2O_5	0.0	%	Acid digestion
Fe	47.33	mg dm^{-3}	Mehlich-1	K_2O	0.2	%	Acid digestion
Mn	2.44	mg dm^{-3}	Mehlich-1	CaO	0.0	%	Acid digestion
Zn	0.28	mg dm^{-3}	Mehlich-1	TiO	0.5	%	Acid digestion
Al	0.45	$cmol_c$ dm^{-3}	KCl (1 mol L^{-1})	Fe_2O_3	3.3	%	Acid digestion
Al + H	2.37	$cmol_c$ dm^{-3}	SMP				

[1] According to Boldrin et al. (2013).

Table S2 - Lines used to determine the elements with ICP-OES and evaluate the accuracy by analyzing Tomato Leaves SRM1573a.

Element or Nutrient	λ (nm)	Certified[a] (mg kg^{-1})	Found[b] (mg kg^{-1})	% recovery[c]
S	180.731	9600	9331 ± 383.37	97
P	178.287	2161 ± 26	2082 ± 67.69	96
K	769.896	26760 ± 480	29546 ± 786.01	110
Ca	315.887	50450 ± 550	49700 ± 1117	99
Mg	279.079	1200	11631 ± 326.75	97
Fe	373.486	367.5 ± 4.3	360.97 ± 19.45	98
Cu	324.754	4.70 ± 0.14	3.61 ± 0.31	77
Zn	213.856	30.94 ± 0.55	30.24 ± 1.63	98
Mn	403.076	246.3 ± 7.1	270.80 ± 7.27	110

[a] Results for Tomato Leaves SRM1573a represented as mean ± confidence interval, informative value. [b] Mean ± standard error of the mean. Average of four determinations. [c] Average of four determinations.

Table S3 - Analysis of variance of sorghum cultivated in the greenhouse and fertilized with different Se sources and doses.

Sources of variation	Mean Square						
	Factorial *vs* Control	Factorial *vs* Additional	Doses	Sources	Doses* Sources	Residue	CV (%)
Grain yield	3.74^{ns}	24.04*	0.68^{ns}	12.87^{ns}	3.15^{ns}	3.37	8.10
Dry mass root	5.04^{ns}	0.06^{ns}	1.14^{ns}	1.35^{ns}	2.21^{ns}	1.70	16.99
Se content in grain	80.33**	1625.16**	2.47^{ns}	117.29**	61.15**	0.64	17.63
Se content in shoot	97.93**	1893.24**	3.52*	132.89**	51.80**	0.52	14.34
Se content in root	17.98**	26.08**	30.71**	8.34**	2.12**	0.21	13.54
Absorption efficiency	0.004**	0.064**	0.0002^{ns}	0.004**	0.0025**	0.0001	31.09
Se recovery	15.6^{ns}	3107.3**	1532.2**	3268.4**	145.2**	4.60	9.93
Translocation efficiency – root to shoot + grain	11520**	7625**	6982**	5320**	910**	7.00	4.53
Translocation efficiency – shoot to grain	11.5^{ns}	38.7^{ns}	8916**	357**	469**	26.60	12.24
S content in grain	0.004^{ns}	0.02*	0.001^{ns}	0.010*	0.012*	0.003	4.32
S content in shoot	0.0004^{ns}	0.039*	0.0002^{ns}	0.01^{ns}	0.02*	0.007	6.28
P content in grain	0.13^{ns}	0.014^{ns}	0.02^{ns}	0.07^{ns}	0.13^{ns}	0.06	8.27
P content in shoot	0.02^{ns}	0.34**	0.86**	0.44**	0.18**	0.03	9.35
K content in grain	0.005^{ns}	3.24**	0.67**	1.71**	0.27**	0.06	6.90
K content in shoot	2.19^{ns}	72.46**	85.80**	46.69**	15.45^{ns}	7.07	6.46
Mg content in grain	0.05^{ns}	0.001^{ns}	0.01^{ns}	0.06^{ns}	0.05^{ns}	0.03	10.20
Mg content in shoot	0.13^{ns}	0.04^{ns}	0.08^{ns}	0.02^{ns}	0.01^{ns}	0.06	13.68
N content in grain	0.10^{ns}	18.25*	0.39^{ns}	2.81^{ns}	3.57^{ns}	2.91	5.26
N content in shoot	4.57^{ns}	5.84^{ns}	0.17^{ns}	4.72^{ns}	4.06^{ns}	5.55	12.09
Cu content in grain	1.05**	2.90**	0.9570*	3.97**	0.86**	0.13	10.14
Cu content in shoot	0.00004^{ns}	0.11^{ns}	0.002^{ns}	0.84^{ns}	1.70^{ns}	0.64	10.42
Fe content in grain	331.19**	38.70^{ns}	98.97^{ns}	331.82**	569.97**	24.22	8.30
Fe content in shoot	157^{ns}	2061**	2777**	428*	446*	138.37	11.90
Mn content in grain	6.20^{ns}	21.34^{ns}	0.62^{ns}	50.40^{ns}	84.84*	21.35	10.35
Mn content in shoot	51.7^{ns}	842.2^{ns}	6411.1**	1704.7^{ns}	621.2^{ns}	638.0	12.11
Zn content in grain	133.93^{ns}	13.54^{ns}	128.41^{ns}	124.52*	112.57*	36.26	11.39
Zn content in shoot	28.71^{ns}	5.23^{ns}	3.07^{ns}	512.38**	201.47^{ns}	73.50	6.15
Ca content in shoot	0.20^{ns}	0.32^{ns}	6.86**	1.63**	0.21^{ns}	0.21	8.48
MDA	13.70**	0.23^{ns}	11.97**	4.15**	5.96**	0.40	9.57
H_2O_2	0.07*	0.09**	0.11**	0.02^{ns}	0.01^{ns}	0.01	13.86
SOD	38933^{ns}	368524**	68961^{ns}	379226**	127469**	17000	8.92
CAT	0.05^{ns}	1.36**	0.04^{ns}	1.28**	0.05^{ns}	0.04	17.96
APX	38.51^{ns}	3.07^{ns}	21.33^{ns}	314.68**	687.17**	28.72	18.73
Reducing sugars	36.04^{ns}	8.18^{ns}	89.29**	81.45**	59.13**	11.46	17.40
Sucrose	4.43^{ns}	657.64**	40.07^{ns}	2008.17**	74.30^{ns}	60.29	14.23
Starch	3671^{ns}	326^{ns}	14418**	22252**	25886**	1393	18.74

^{ns} – not significant by F-test; * - significant by F-test at $p<0.05$; ** - significant by F-test at $p<0.01$; number of replicates - 4; CV – coefficient of variation (%); Degrees of freedom: factorial *vs* control (F *vs* C)-1, factorial *vs* additional (F *vs* A) -1, doses (D) – 1, sources (S) – 3, doses*sources (D*S) – 3, residue – 30.

ARTIGO 2 – SELENATE FERTILIZATION IN SORGHUM GROWN IN TROPICAL SOILS AND ITS EFFECT ON MINERAL CONTENT AND ANTIOXIDANT METABOLISM

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SELENATE FERTILIZATION IN SORGHUM GROWN IN TROPICAL SOILS AND ITS EFFECT ON MINERAL CONTENT AND ANTIOXIDANT METABOLISM

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Abstract

Since sorghum is the staple food for millions of people, coinciding with areas where selenium (Se) deficiency occurs. Biofortification with Se in sorghum can considerably improve Se intake in humans living in these areas. In this study, the objective was to evaluate the response of sorghum plants to Se biofortification, and the effect of Se foliar application through sodium selenate on grain yield, antioxidant system, and mineral content. In the greenhouse, Se was applied at the rates of 0, 0.125, 0.250 and 0.500 mg plant^{-1} and in the field, Se was supplied at the doses of 0, 10, 20, 40 g ha^{-1} of in the cultivars BM737, BRS310, Enforcer, K200, Nugrain320, Nugrain420, Nugrain430, and SHS410. The highest grain yield was observed in Nugrain420 and Nugrain430 in a greenhouse with 0.250 mg plant^{-1} of Se. In the field, Lavras and Lambari, the highest grain yield occurred with Nugrain430 and Nugrain320 with 20 and 40 g ha^{-1} of Se, respectively. Selenium efficiency absorption in K200 was higher with 0.125 mg plant^{-1} of Se in the greenhouse. However, the field was higher with 20 g ha^{-1} of Se. Selenium improves the antioxidant metabolism and mineral content of the studied cultivars. Therefore, sorghum plants respond positively to the Se foliar application, and the ingestion of sorghum grains biofortified with Se can provide Se for humans and animals.

Keywords: food composition, beneficial elements, field conditions, greenhouse conditions, food security

1. Introduction

Cereals and their derivatives are the primary sources of energy for animals and humans. Sorghum (*Sorghum bicolor* L. Moench) is the fifth largest cereal crop globally, after rice, wheat, barley, and maize. It is the staple food for nearly 500 million people (Raswan et al., 2021). Sorghum is an important component of animal nutrition in the United States, Australia and South America (Fornasieiri Filho and Fornasieiri, 2009). The importance of this cereal is due to the fact that its grains, stems and leaves can be used for forage and feed (Rooney et al., 2007). The world production of sorghum in 2019 reached 40 million hectares and about 57.9 million tons. In the same year, Brazil produced 2.7 million tons on 818,300 hectares (FAOSTAT, 2020). Sorghum is a globally competitive crop because it has agronomic advantages in adverse environments, drought resistance, high productivity, low mineral nutrition requirements, and low production costs (Adebowale et al., 2020).

Sorghum grains are rich in macronutrients, micronutrients, and bioactive compounds. They also have high nutritional value and antioxidants, anti-obesity, anti-diabetic, anti-cardiovascular, anti-inflammatory, antimicrobial, and anticancer activity (Raswan et al., 2021). Since sorghum protein does not cause autoimmune, allergic reactions, sorghum is recommended to be safe for patients with celiac disease (Ofosu et al., 2020). Health care has become more assertive in recent years. People seek foods that supply their daily needs for vitamins and minerals and avoid illnesses caused by a lack of certain vital substances (Delaqua et al., 2021).

The role of Se in the antioxidant process contributes to the normal functioning of the immune and thyroid system (Fairweather-Tait et al., 2011), and its importance for humans and animals have already been recognized (Terry et al., 2000). It is also considered a chemopreventive agent because adequate nutrition with this element can reduce cancer risk (Serrano-Sandoval et al., 2019). Small amounts of selenium are needed to maintain good health, but excessive intake of this nutrient can cause health problems (Cozzolino, 2000).

Selenium biofortification can be done by Se supplying via soil and/or leaves. Foliar fertilization is more efficient than soil fertilization because Se absorption occurs more effectively, as there is no interference from the soil matrix (Kopsell et al., 2009). In addition, foliar Se supplementation from plants requires a smaller amount of Se salts than when applied via soil, which generates a reduction in costs (Djujic et al., 2000). Furthermore, foliar fertilization is less harmful to the environment under field-grown conditions, preventing the elements accumulation in the soil and causing pollution (Lyon, 2018). Several authors have

reported the effectiveness of foliar biofortification with selenium in various crops (Schiavon et al., 2016; Lara et al., 2019; Silva et al., 2020; Delaqua et al., 2021; Lessa et al., 2020).

However, the toxicity limit, the impact on the content of other minerals in the plant, and the chemical Se form must be considered to avoid a decline in crop yield (Longchamp et al., 2013). Plant species differ in their ability to concentrate and accumulate of Se, in the Se distribution in plant parts and tissues, and in its affinity to absorb Se rather than sulfur (S). Excessive amounts of Se can cause phytotoxicity to plants through the use of high doses or excessive exposure time (Hawrilak-Nowak et al., 2015). In the level of phytotoxicity, Se acts as a pro-oxidant in plants, increasing the hydrogen peroxide (H_2O_2) concentration and inducing lipid peroxidation (Ríos et al., 2008). In this context, the objective was to evaluate sorghum response to biofortification with Se, and the effect of Se foliar application through sodium selenate on the grain yield, antioxidant system, and mineral content.

2. Material and Methods

2.1.Growing conditions

The experiments were conducted from February to August 2019, with one experiment in a greenhouse at the Department of Soil Science at the Federal University of Lavras and two field experiments located at the Experimental Farm of the Agricultural Research Company of the State of Minas Gerais (EPAMIG) in Lambari; and the Center for Scientific and Technological Development in Agriculture – Fazenda Muquém/UFLA in Lavras, both cities located in the State of Minas Gerais, Brazil (Fig. 1). The climate in Lavras and Lambari, according to the Köppen climate classification, is Cwa (Dantas et al., 2007; Martins et al., 2018), which presents characteristics such as mesothermal (hot temperate or subtropical), with winter drought (with precipitation in the driest month <60 mm, with the temperature in the coldest month ranging between ≥ -3°C and <18°C) and hot summer (with temperature in the hottest month without 22°C).

In the greenhouse, the dystrophic Red-Yellow Latosol (Santos et al., 2018) was used, a sandy clayey loam texture, collected in the 0 – 20 cm layer, corresponding to the Typic Haplustox (loam) in the Soil Taxonomy (Soil Survey Staff, 1999). In Lavras, the soil in the experimental area was the Dystrophic Red-Yellow Latosol (Santos et al., 2018), corresponding to an Oxisol according to the Soil Taxonomy (Soil Survey Staff, 1999). In Lambari, the soil of the experimental area is classified as a Melanic Gleysol (Santos et al., 2018) corresponding to a Histosol according to the Soil Taxonomy (Soil Survey Staff, 1999).

The soils in this area have a history of agricultural cultivation. A soil sample was taken from the experimental areas before the implementation of the experiments. The soil samples were air-dried, sieved with a 4 mm mesh, and characterized by their main physical and chemical properties, according to Teixeira et al. (2017) (Table 1 – supplementary material).

2.1.1. Greenhouse-grown conditions

Pots with 5 dm^{-3} of soil were used. Based on the chemical analysis of the soil, the calculation of liming was done by the method of neutralization of Al^{+3} and the increase in the Ca^{+2} and Mg^{+2} contents (Ribeiro et al., 1999). Liming was performed using 2.725 mg CaO_3 and 0.649 mg MgO_3 per dm^{-3} of soil. After 30 days of incubation of the soil with moisture close to 60% of the total pore volume (VTP), the basic fertilization for planting was carried out, which consisted of 200 mg of P + 90.3 mg of N ($NH_4H_2PO_4$), 108.4 mg of K + 44.47 mg of S (K_2SO_4), 5 mg of Zn + 2.44 mg of S ($ZnSO_4.7H_2O$), 1.5 mg of Cu + 0.75 mg of S ($CuSO_4.5H_2O$), 4.0 mg of Mn + 2.33 mg of S ($MnSO_4.H_2O$), 0.5 mg of B (H_3BO_3) and 0.1 mg of Mo ($[NH_4]_6Mo_7O_{24}.4H_2O$) per dm^{-3} of soil.

Before sowing, seeds were treated with Standak Top® and Cruiser® as recommended. Subsequently, ten sorghum (*Sorghum bicolor* L.) seeds were sown per pot, and ten days after seedling emergence, thinning was done, leaving two seedlings per pot. During the sorghum cultivation period, fertilization was carried out to meet the nutritional demand of the plants. After thinning were applied: 0.25 mg of B (H_3BO_3), 0.75 mg of Cu ($CuSO_4.5H_2O$), 2 mg of Mn ($MnSO_4.H_2O$), 0.05 mg of Mo ($[NH_4]_6Mo_7O_{24}.4H_2O$), 2.5 mg of Zn ($ZnSO_4.7H_2O$). Furthermore, after 50 days of cultivation, 4 mg of Mn ($MnSO_4.H_2O$) and 5 mg of Fe (EDDHA iron chelate) were applied.

The macronutrients were divided into 14 applications during the cycle, and the 1st application consisted of 25 mg of Ca + 17.5 mg of N ($Ca(NO_3)_2$), 30 mg of K and 8.37 mg of N (nitrate commercial potassium), 4.13 mg of N (NH_4NO_3). The 2nd application consisted of 25 mg of Ca + 17.5 mg of N ($Ca(NO_3)_2$), 30 mg of K and 8.37 mg of N (potassium nitrate - commercial), 4.13 mg of N (NH_4NO_3), and 12 mg of Mg ($MgSO_4$). The 3rd, 4th, 5th, 6th, 7th, 11th and 12th applications consisted of 50 mg of K + 13.95 mg of N (potassium nitrate - commercial) and 36.05 mg of N (NH_4NO_3). The 8th, 9th, 10th, 13th and 14th applications consisted of 30 mg of K + 8.37 mg of N (potassium nitrate - commercial) and 21.82 mg of N (NH_4NO_3).

The sodium selenate (Na_2SeO_4) diluted in a 0.5% surfactant solution (Assist®) was

used to prepare the following Se doses: 0.125, 0.250, and 0.500 mg plant^{-1}, and two applications were made. The first foliar application of Se was carried out when the plants were in the flowering stage and the second stage of grain filling. The control treatment received only deionized water containing the surfactant. The foliar sprays in the greenhouse were carried out with a manual sprayer with the previous compression and applied an amount of 60 mL pot^{-1}. Greenhouse temperatures were maintained at 28 ± 5°C during the day with an automatic control system. The pots were irrigated, and the soil water content was adjusted daily close to the field capacity.

2.1.2. Field-grown conditions

Before sowing, seeds were treated with Standak Top® and Cruiser® as recommended. Sowing was done in conjunction with planting fertilization, which consisted of 350 kg ha^{-1} of commercial fertilizer formula NPK 8-28-16 (N-P_2O_5-K_2O). After 40 days, the top dressing was done with 480 kg ha^{-1} of the formula NPK 23-00-11 (N-P_2O_5-K_2O) with 2.7% of S. Solutions with Se for in the field spraying were prepared in the same way as solutions applied in the greenhouse, and used at the same vegetative stage.

The sodium selenate (Na_2SeO_4) diluted in a 0.5% surfactant solution (Assist®) was used to prepare the following Se doses: 10, 20, and 40 g ha^{-1}. In the field, spraying was carried out with a pressurized pump coupled to a carbon dioxide container and was applied 500 mL in each experimental plot. A 2 m long bar was connected to the pump for uniform application. Foliar applications were made in the morning to provide more excellent foliar absorption of Se.

2.2. Biochemical analysis

Sampling was carried out on the fifth day after the second Se application. The V2 leaves were collected immediately conditioned in N_2 liquid and stored at -80°C for biochemical analysis. Hydrogen peroxide (H_2O_2), lipid peroxidation (MDA), proteins, and the activities of the superoxide dismutase (SOD, EC: 1.15.1.1), catalase (CAT, EC: 1.11.1.6), and ascorbate peroxidase (APX, EC: 1.11.1.11) were quantified.

2.2.1. Hydrogen peroxide (H_2O_2) and lipid peroxidation (MDA)

The total of 0.4 g of the fresh mass leaves was macerated in N_2 liquid and PVPP (polyvinylpolypyrrolidone), homogenized in 1.5 mL of 0.1% (w/v) trichloroacetic acid (TCA)

and centrifuged at 12000 g for 15 minutes at 4°C. The hydrogen peroxide (H_2O_2) was quantified from the obtained extract as described in Velikova et al. (2000), based on a standard curve with known H_2O_2 concentrations. Lipid peroxidation was determined as described by Buege and Aust (1978), and the results were expressed in nanomoles of malondialdehyde (MDA) per gram of fresh mass.

2.2.2. Extraction, quantification of antioxidant enzymes and protein

The total of 0.2 g of the fresh mass leaves was macerated in N_2 liquid with PVPP (polyvinylpolypyrrolidone) and homogenized with 1.5 mL of buffer solution ((0.1 mol L^{-1} potassium phosphate (pH 7.8), 0.1 mmol of EDTA (pH 7.0) and 0.01 mol L^{-1} ascorbic acid)). Then, 13000 g were centrifuged for 10 minutes at 4°C, and the supernatant was collected and stored at -20°C (Biemelt et al., 1998). The supernatant was collected and used for the enzymatic analysis of superoxide dismutase (SOD) (Giannopolitis and Ries, 1977), catalase (CAT) (Havir and Mchale, 1987), ascorbate peroxidase (APX) (Nakano and Asada, 1981), and protein (Bradford, 1976).

2.3. Grain yield and grain weight

The sorghum grains produced were harvested and weighed to determine grain yield. In the greenhouse, grain yield was determined by collecting the grain produced by plants per pot and divided by the number of plants. While in the field, grain yield was evaluated by harvesting sorghum plants from the two central rows (useful plot). According to the Seed Analysis Rule (MAPA, 2009), grain moisture was determined, and grain yield was converted into dry weight by a correction of 13% moisture. The weight of a sample of 1000 grains from each plot was also determined, and it was also converted into dry weight by a correction of 13% moisture.

2.4. Sample digestion procedure

The plants were separated into grains and shoots and then placed in a cross-airflow at a temperature of ±60°C until they reached constant weight (after ±72 h). After drying, the plants were ground, and then 0.5 g of each sample was taken for digestion by the 3051A method described by the United States Environmental Protection Agency (USEPA, 2007). Each aliquot was digested with 5 mL of HNO_3 in PTFE Teflon ® tubes (CEM Corporation, Matthews, NC, USA). The extract was left to stand overnight at room temperature, and

digestion was carried out the following day. For this, the flasks were hermetically sealed and placed in a microwave (CEM brand, model Mars-5), with a temperature adjusted to 175°C and a controlled pressure of 0.76 MPa for 15 minutes. After digestion, the extracts were cooled to room temperature. Then, the final volume of the extract was supplemented with an additional 5 mL of deionized water, with the final volume being transferred to flasks and stored at 5°C until analysis.

2.5. Macronutrients and micronutrients determination

The determinations of the S, P, K, Ca, Mg, Fe, Cu, Zn, and Mn contents were carried out by optical emission spectrometry with inductively coupled plasma (ICP-OES), brand Spectro, model Blue (Germany), with correction background. The operating parameters and the sample introduction system were as indicated by the manufacturer: plasma power of 1400 W, cooling gas flow of 12 L min^{-1}, the auxiliary gas flow of 0.8 L min^{-1}, and gas flow in the nebulizer 0.85 L min^{-1}. The gas used was argon with a purity ≥ of 99.99%. The determinations were carried out in a multi-elemental way and, thus, standard solutions were prepared using aliquots of standard stock solutions of 1000 mg L^{-1} of the elements under study. Dilutions for all solutions were made with the solvent used in the procedure. The calibration curve for the proposed method contained at least five standards of known concentration. The spectral line for each determining element is shown in Table 2 - supplementary material. A sample of standard reference material (Tomato Leaves SRM1573a) for plant material was included in each batch for quality control purposes, along with a blank sample. The accuracy of the analytical procedure was verified by analyzing the standard reference material and the results found are following the certified values and the recovery ≥ of 77%. The total N content was determined by sulfuric digestion and Kjeldahl distillation (Malavolta et al. 1997). It also used a sample of Tomato Leaves standard reference material (SRM1573a) in each digestion batch for quality control purposes, along with a blank sample. The average N recovery in this material was 118%.

2.6. Selenium (Se)

Selenium in the digested samples were analyzed by GFAAS (Atomic Absorption Spectrometry with Zeeman background correction and EDL lamp for Se; AAnalyst ™ 800 AAS, Perkin Elmer). A standard solution containing 1 g kg^{-1} Se (98% purity, Fluka, Buchs, Switzerland) was used to prepare the calibration curve. A sample of standard reference

material (White Clover - BCR 402, Institute for Reference Materials and Measurements, Geel, Belgium) for plant material was included in each digestion batch for quality control purposes, along with a used blank sample to calculate the limits of detection and quantification. The mean Se recovery in this standard reference material (white clover) was 92.9% (n = 10, [Se] = 6.57 mg kg^{-1}).

The limits of detection (LOD) and quantification (LOQ) were established using ten blank extracts following the same procedure adopted with the samples. Values were calculated three and ten times the standard deviation of ten blank extracts to determine LOD and LOQ, respectively (Khan et al., 2013). In the analysis of the materials under study, the LOD was 83.9 µg kg^{-1} of extract Se, and the LOQ was 279.8 µg kg^{-1} of extract Se.

2.7. Efficiency nutritional of Se

To study the Se efficiency use by sorghum plants, mathematical expressions of nutritional efficiency concepts proposed by several researchers were applied. Selenium uptake (SeU) was expressed in mg $plant^{-1}$ for the result of the experiment conducted in the greenhouse because production is quantified in g $plant^{-1}$, and it was expressed in mg kg^{-1} for the result of the field experiments because productivity is expressed in kg ha^{-1} (Ducsay et al., 2016; Lara et al., 2019). Selenium absorption efficiency (SeAE) was expressed in % (Lara et al., 2019). The equations were described below.

$$SeU = \text{Se content in grain (mg } hg^{-1}) \text{ x grain yield} \quad \text{(Eq. A1)}$$

$$SeAE = \{(SeU_{treated} - SeU_{control}) * 100\} / \text{Se supply (g } ha^{-1}) \quad \text{(Eq. A2)}$$

2.8. Experimental Design

The experiment was carried out in a greenhouse, in a completely randomized design in a 4 x 8 factorial scheme, with four Se doses (control - without Se application; 0.125, 0.250, and 0.500 mg $plant^{-1}$ of Se by sodium selenate) and eight cultivars (BM737, BRS310, Enforcer, K200, Nugrain320, Nugrain420, Nugrain430 and SHS410) and four replicates, totaling 128 experimental plots. The cultivars used in this study are commercial cultivars planted in Brazil with high grain quality and no tannin (Table 3 – supplementary material). Details about the patent and maintainer of the cultivar can be found in Brazil (2020). The experiment under field conditions was installed in a randomized block design, with the same factorial adopted in the greenhouse. With four Se doses: control - without Se application; 10, 20, and 40 g ha^{-1} of Se by sodium selenate. The plot consisted of four rows of 4 m linear,

spaced 0.6 m apart, totaling 9.6 m² per plot. The two lateral lines were considered as borders. The useful area considered the two central lines, and 0.5 m was discarded at each end, totaling 3.6 m² for grain harvesting.

2.9. Statistical analysis

Initially, the data obtained in the greenhouse were analyzed for normality by the Shapiro-Wilk test ($p \geq 0.05$), homoscedasticity by the Barlet test ($p \geq 0.05$), and the independence of residues by the Durbin Watson test ($p \geq 0.05$). Then they were submitted to an analysis of variance. Data from field experiments were introduced to an individual analysis by experiment in the same way as in the greenhouse. After accepting the assumptions for the joint analysis of experiments, a joint analysis of variance was performed with the two field experiments, considering simultaneously all the experiments developed in Lavras and Lambari (Pimentel-Gomes, 2009). Means were compared using the Tukey test $p \leq 0.05$.

The simple linear relationship between the variables was carried out through a simple Pearson's correlation. According to Cao et al. (1999), data were standardized before clustered samples because the variables were measured in different units. Principal component analysis (PCA) was performed to report the variation in Se doses for each cultivar. This analysis allowed for the characterization of the variables that most discriminate concerning structural characteristics in each treatment. Thus, the initial set of variables started to be described by two new orthogonal latent variables, making it possible to locate them in two-dimensional figures (Hair Junior et al., 2009). Statistical analysis and graphics were performed using the R software (R Core Team, 2021).

3. Results

The analysis of variance (ANOVA) of the experiment in the greenhouse (Table 4 – supplementary material) revealed that the response of the sorghum plants was dependent on the interaction between cultivars and Se doses (C x D) for Se content in the grain and the shoot, Se uptake (SeU), Se absorption efficiency (SeAE), H_2O_2; MDA; CAT; APX; protein in the enzyme extract; grain yield; N, S, P, Ca, Mg, Fe, Zn, Cu, Mn content in grain; N, S, P, K, Ca, Mg, Fe, Zn, Cu content in the shoot. For SOD, grain weight, K content in grain, and Mn content in the shoot, the effect of cultivars was independent of Se fertilization.

For the field experiment, the ANOVA of the joint analysis (Table 5 – supplementary material) indicated a significant difference ($p \leq 0.01$) between the locations (Lavras and

Lambari) for all variables under study. The interaction was significant between cultivars, Se doses, and locations (C x D x L) for grain yield; Se, S, P, K, Ca, Mg, Fe, Zn and Cu content in grain; Se, N, P, Ca, Fe, Zn, Cu, Mn content in the shoot, Se uptake, and Se absorption efficiency. For grain weight and S content in the shoot, the double interactions of cultivars and locations (C x L) and Se doses and locations (D x L) were affected. Only the dual interaction among cultivars and locations (C x L) was significant for: N and Mn content in grain, and K and Mg content in the shoot.

3.1. Antioxidant metabolism

The effect of the Se application changed the hydrogen peroxide content (H_2O_2) (Fig. 2A) in the cultivars BM737 and K200, with a lower H_2O_2 content when 0.125 mg plant^{-1} of Se was applied. The cultivar K200 with application of 0.125 mg plant^{-1} of Se had lower H_2O_2 content 31.3, 55.6 and 54.2% than the control treatment, 0.250 and 0.500 mg plant^{-1} of Se respectively. Selenium application of 0.125 mg plant^{-1} increased lipid peroxidation (MDA) (Fig. 2B) by 25.2, 29.5 and 31.9% when compared with the control treatment, 0.250 and 0.500 mg plant^{-1} of Se, respectively in BRS310. Selenium application of 0.125, 0.250, 0.500 mg plant^{-1} reduced the MDA by 36.1, 23.7 and 43.9%, respectively, in relation to the control treatment in SHS410. Selenium application of 0.500 mg plant^{-1} provided the lowest MDA in BRS310, Enforcer, K200, Nugrain430, and SHS410 compared with the application of 0.250 mg plant^{-1}.

The catalase activity (CAT) (Fig. 2C) was higher with 0.500 mg plant^{-1} of Se, which resulted in increments of 26.3, 19.4 and 24.3% compared with the control, 0.125 and 0.250 mg plant^{-1} of Se, respectively in BRS 310. Selenium application of 0.500 mg plant^{-1} also had higher CAT activity with the cultivars BM737 and Nugrain420. Selenium application of 0.125 mg plant^{-1} provided the highest activity of ascorbate peroxidase (APX) (Fig. 2D) with the cultivar SHS410, which was higher 15.0, 17.0 and 49.8% in relation to the treatment control, 0.250 and 0.500 mg plant^{-1} of Se, respectively. However, selenium application of 0.500 mg plant^{-1} provided greater APX activity in cultivars BRS310, Enforcer, K200, Nugrain320, and Nugrain430. The application of 0.500 mg plant^{-1} of Se provided higher protein content in the enzymatic extract (Fig. 2F) in cultivars BRS310, SHS410, and Nugrain430. However, the application of 0.250 and 0.500 mg plant^{-1} of Se increased the protein content of the Enforcer.

3.2. Selenium (Se) and efficiency nutritional

The foliar application of Se increased the Se content in the grain and in the shoot with all Se doses applied in both growing conditions, i.e., greenhouse and field. Selenium content in the control treatment was not possible to determine, since the Se contents were below the detection and quantification limit. In the greenhouse, the highest contents occurred with the Se dose of 0.500 mg plant^{-1} and the most responsive cultivar was Nugrain430. Selenium content in the grain was higher with 0.500 mg plant^{-1} of Se, which presented increments of 65.9 and 42.1% in relation to 0.125 and 0.250 mg plant^{-1} of Se in Nugrain430 (Fig. 3A). Selenium content in the shoot with Se dose of 0.500 mg plant^{-1} in Nugrain430 was 70.8 and 51.9% higher than 0.125 and 0.250 mg Se plant^{-1} of Se, respectively (Fig. 3B).

In the field, Se contents increased with increasing Se doses applied. In Lambari, the highest Se content in the grain occurred with 40 g ha^{-1} of Se with BRS310, which had increments of 74.8 and 44.8% in relation to 10 and 20 g ha^{-1} of Se, respectively (Fig. 4A). In Lavras, the highest Se content in the grain occurred with 40 g ha^{-1} of Se with the Enforcer, which was 54.4 and 71.0% higher than the 10 and 20 g ha^{-1} of Se. Selenium application of 40 g ha^{-1} promoted the highest levels of Se in the shoot. In Lambari, the Se dose of 40 g ha^{-1} promoted increments of 44.8 and 65.2% compared with 10 and 20 g ha^{-1}, respectively in BRS310 (Fig. 4B). In Lavras, the Se dose of 40 g ha^{-1} increased by 54.6 and 78.3% compared with 10 and 20 g ha^{-1} of Se, respectively, in Nugrain420.

In the greenhouse, the absorption of selenium (SeU) (Fig. 3C) was higher with 0.500 mg plant^{-1} in Nugrain430, which was 63.2% and 30.2% higher than 0.125 and 0.250 mg plant^{-1} of Se respectively. However, the Se doses of 0.250 and 0.500 mg plant^{-1} did not differ in cultivars BM737, BRS310, K200 and Nugrain420. In the field, SeU (Fig. 4C) was higher with the application of 40 g ha^{-1} of Se in all cultivars studied. Selenium dose of 40 g ha^{-1} had increments of 81.1 and 59.4% in relation to 10 and 20 g ha^{-1} of Se in Nugrain320 in Lambari. In Lavras the highest SeU occurred with 40 g ha^{-1} of Se with K200, which was higher 75.4 and 38.6% compared with 10 and 20 g ha^{-1} of Se, respectively. However, the application of 20 and 40 g ha^{-1} of Se did not differ significantly in BM737 in Lambari and in Nugrain320 in Lavras.

The absorption efficiency of selenium (SeAE) by sorghum plants In the greenhouse (Fig. 3D) was higher with the Se dose of 0.125 mg plant^{-1} in K200, with increments of 13.8 and 52.9% with 0.250 and 0.500 g ha^{-1} of Se, respectively. Selenium dose of 0.500 mg plant^{-1} reduced SeAE in all cultivars. In the field, the applied Se doses did not differ significantly in

cultivars in Lambari. In Lavras, the highest SeAE occurred with the application of 10 g ha^{-1} of Se in BRS310, which was 17.7 and 21.7% higher than the application of 20 and 40 g ha^{-1} of Se, respectively (Fig .4D).

3.3. Grain yield

Selenium fertilization of sorghum plants grown in a greenhouse (Fig. 3E) had increases in grain yield with the application of 0.250 mg $plant^{-1}$ of Se in Nugrain420, which was 17.3 and 17.7% higher than when applied 0.125 and 0.500 mg $plant^{-1}$, respectively. Selenium doses of 0.500 mg $plant^{-1}$ also reduced the grain yield of BM737, BRS310, Enforcer, and Nugrain430. For sorghum plants grown in the field (Fig. 4E), the Se application influenced the grain yield of BRS310, Enforcer and K200 in Lambari. And Selenium dose of 10 g ha^{-1} resulted in higher Enforcer grain yield, with increments of 24.5, 19.8 and 6.5% in relation to the control treatment, 20 and 40 g ha^{-1} of Se, respectively. In Lavras, the Se dose of 20 g ha^{-1} in K200 increased by 19% compared with 10 g ha^{-1} of Se.

3.4. Macronutrient content

In the greenhouse, the N content in the grain (Fig. 5A) was higher with 0.500 mg $plant^{-1}$ of Se, however it did not differ significantly from when 0.250 mg $plant^{-1}$ of Se was applied in the BRS310. Selenium dose of 0.500 mg $plant^{-1}$ also provided higher N contents in grains of BM737, Enforcer, Nugrain420, and Nugrain430. The Enforcer with 0.250 mg $plant^{-1}$ of Se had lower levels of 10.7, 23.1 and 20.8% compared with the control treatment, 0.125 and 0.500 mg $plant^{-1}$ of Se, respectively. In the field, the N content in grain and shoot was not influenced by Se doses.

Application of Se influenced the S content in the grain (Fig. 5C) only in cultivars Enforcer and Nugrain420 in the greenhouse. Selenium dose of 0.125 mg $plant^{-1}$ provided increments of 13.7% in relation to the control treatment in Enforcer. In this cultivar, the increase in Se doses decreased the S content in the grain, with a reduction of 12.1 and 7.1% with 0.250 and 0.500 mg $plant^{-1}$ in relation to 0.125mg $plant^{-1}$ of Se. The S content in the shoot (Fig. 5D) was affected by the Se application only in the enforcer cultivar, which presented a response similar to the S content in the grain. The Enforcer with 0.125 mg $plant^{-1}$ of Se was 33.1% higher than the control treatment and 27.1% lower than 0.250 mg $plant^{-1}$ of Se.

In the field, the effect of Se application on Se content in the grain (Fig. 6D) was

different among cultivars. In Lambari, the Se dose of 40 g ha^{-1} had increments of 7.9, 11.3 and 7.6% in relation to the control treatment, 10 and 20 g ha^{-1}, respectively, with the cultivar Enforcer. However, at Se dose 40 g ha^{-1} reduced 9.7, 5.7 and 7.2% compared with the control treatment, 10 and 20 g ha^{-1} of Se, respectively, BRS310. In Lavras, Se doses did not differ significantly between cultivars for Se content in grains.

In the greenhouse, Se dose of 0.125 mg $plant^{-1}$ provided the highest P content in the grain in BRS310, which was 21.3, 13.4 and 12.4% higher than the control treatment, 0.250 and 0.500 mg $plant^{-1}$ Se (Fig. 5E). The P content in shoot when applied 0.125 mg $plant^{-1}$ increased by 22.5, 27.6 and 40.3% compared with the control treatment, 0.250 and 0.500 mg $plant^{-1}$ of Se, in Nugrain320. The dose of 0.125 mg $plant^{-1}$ increased by 35.5% compared with the control treatment in Enforcer (Fig. 5F). In field experiments, the P content in grain and shoot was little influenced by Se doses (Fig. 6B and 6C). The doses 10, 20 and 40 g ha^{-1} of Se provided increments in the P content of the grain and shoot only in the cultivar Nugrain430 in Lambari. At doses of 10 g ha^{-1} of Se reduced the P content in grain and shoot of Nugrain320, Nugrain430 and SHS410 compared with the control treatment.

In the greenhouse, the K content in the grain was not significantly influenced with the applied Se doses. And the main effect of the Se doses on the K content in the shoot (Fig. 5G) occurred in the BM737 and the Enforcer. In the field, the highest K contents in the grain (Fig. 6D) occurred with 40 g ha^{-1} of Se, which increased 12.5, 20.5 and 29.5% compared with the control treatment, 10 and 20 g ha^{-1} of Se, respectively, on the Nugrain320 in Lambari. In Lavras, the 20 g ha^{-1} of Se increased the K contents in the grain by 18.4 and 11.3% compared with 10 and 40 g ha^{-1} in Nugrain320.

The Mg content in the grain (Fig. 5H) of sorghum cultivated in a greenhouse had the most significant increase at the dose of 0.125 mg $plant^{-1}$ of Se , which was 31.8, 20.5 and 24.4% higher than the control treatment, 0.250 and 0.500 mg $plant^{-1}$ of Se, respectively in BRS310. The Mg content in shoot (Fig. 5I) was higher at 0.250 mg $plant^{-1}$ of Se, which increased by 25.9% compared with 0.500 mg $plant^{-1}$ in Nugrain420. In the field, the main effects on the Mg contents in the grains were with 20 g ha^{-1} of Se with increments of 19.6 and 19.6% compared with the control treatment and 10 g ha^{-1} of Se, respectively Nugrain320 in Lambari. In Lavras, 40 g ha^{-1} of Se increased by 33.5 and 28.7% compared with 10 and 20 g ha^{-1} of Se in SHS410 and Nugrain320, respectively (Fig. 6E).

The most significant increase in Ca content in the grain occurred with BRS310 with 20 g ha^{-1} of Se, 24.0, 26.0, and 23.0% higher than the control treatment, 10 and 40 g ha^{-1} of Se,

respectively, in the greenhouse. Selenium doses did not differ significantly with Nugrain320 and Nugrain420 (Fig. 5K). The Ca content in the shoot did not differ between the Se doses to BM737, Enforcer, K200, Nugrain320, and SHS410 in the greenhouse. BRS310 and Nugrain430 had contrasting results with the 40 g ha^{-1} of Se. At 40 g ha^{-1} of Se it increased the Ca content of Nugrain430 and reduced it in BRS310. (Fig. 5L).

In the field, Ca content in the grain in Lambari was higher with Nugrain420 with 40 g ha^{-1}, which was 34.0, 31.3, and 18.1% higher than the control treatment, 10 and 20 g ha^{-1} of Se, respectively. In Lavras, the 40 g ha^{-1} of Se also promoted the highest Ca contents in the grain. The Enforcer with 40 g ha^{-1} of Se had 53.4, 43.8, and 61.8% increments concerning the control treatment, 10 and 20 g ha^{-1} of Se, respectively. Furthermore, the Nugrain320 with 40 g ha^{-1} of Se had 35.2, 29.3, and 46.3% compared with the control treatment, 10 and 20 g ha^{-1} of Se, respectively (Fig. 6K). The Ca content in the shoot, in Lambari, the highest contents occurred with BRS310 compared with the other cultivars within each Se dose. However, in the Se doses, there was no significant difference. In Lavras, it generally had higher Ca contents than in Lambari. The main difference between the Se doses was 16.1% between 10 g ha^{-1} of Se concerning 40 g ha^{-1} of Se in the SHS410 (Fig. 6L).

In the greenhouse, the most significant increase in Ca content in the grain (Fig. 5J) occurred with 0.250 mg $plant^{-1}$ of Se, which was 24.0, 26.0 and 23.0% higher than the control treatment, 0.125 and 0.500 mg $plant^{-1}$ Se, respectively, in BRS310. The Ca content in the shoot (Fig. 5K) with the Se dose of 0.500 mg $plant^{-1}$ provided contrasting results, as it caused an increase in the Ca content in Nugrain430 and reduced the Ca content in BRS310 and Nugrain420 when compared with the other doses applied. In the field, the Ca content in the grain in Lambari was higher with 40 g ha^{-1}, which was 34.0, 31.3 and 18.1% higher than the control treatment, 10 and 20 g ha^{-1} of Se, respectively, on the Nugrain420. In Lavras, 40 g ha^{-1} of Se also promoted the highest contents of Ca in the grain. The Enforcer with 40 g ha^{-1} of Se had increments of 53.4, 43.8 and 61.8% in relation to the control treatment, 10 and 20 g ha^{-1} of Se, respectively. Furthermore, Nugrain320 with 40 g ha^{-1} of Se had 35.2, 29.3 and 46.3% compared with the control treatment, 10 and 20 g ha^{-1} of Se, respectively (Fig. 6F).

3.5. Micronutrient's content

The Fe content in the grain had a varied response in relation to the doses of Se applied In the greenhouse (Fig. 7A). The highest Fe contents in the grain occurred with Se 0.125, 0.250, 0.250 and 0.500 mg $plant^{-1}$ doses in BRS310, Nugrain320, Nugrain420 and

Nugrain430 respectively. The Fe content in shoot (Fig. 7B) was higher with 0.250 mg plant^{-1} of Se in Nugrain420, which was 30.1, 29.9 and 30.4% higher than the control treatment, 0.125 and 0.500 mg plant^{-1} of Se, respectively. The dose of 0.250 mg planta^{-1} of Se also generated increases in relation to the other doses of Se in Enforcer, Nugrain320, Nugrain420 and SHS410. The doses 0.125 and 0.500 mg plant^{-1} increased the levels in BM737 and K200, respectively, in relation to the other doses of Se applied.

In the field, the Fe content in the grain (Fig. 8A) with the dose of 40 g ha^{-1} of Se had an increase of 22.6% compared with 10 g ha^{-1} of Se in the Enforcer. BRS310 with 40 g ha^{-1} of Se reduced the Fe content in the grain by 15.5% compared with the control treatment, in Lambari. In Lavras, the dose of 40 g ha^{-1} of Se increased the Fe content by 14.9% than that of 10 g ha^{-1} of Se in SHS410. The Fe content in BM737 with 40 g ha^{-1} of Se was 22.5% lower than 10 g ha^{-1} of Se . The main differences observed in the Fe content in the shoot (Fig. 8B) occurred with 20 g ha^{-1} of Se, which presented increments of 16.5 and 22.2% in relation to the control treatment and 40 g ha^{-1} of Se , respectively, with the BRS310 in Lambari. The dose of 20 g ha^{-1} of Se had increments of 27.7 and 33.9% compared with the control treatment and 10 g ha^{-1} of Se, respectively, with Enforcer in Lavras.

In the greenhouse, the Se doses influenced only the Zn contents in the grain of cultivars BM737 and BRS310 (Fig. 6C). The highest Zn content in the grain occurred with 0.250 mg plant^{-1} of Se, which provided an increase of 27.0% in the control treatment in BM737. The dose of 0.500 mg plant^{-1} in BM737 provided increases of 23.5% in relation to the control treatment. BRS310 had higher Zn content with 0.125 mg plant^{-1} of Se, which had an increase of 23.7% compared with the control treatment. The Zn contents in the shoot (Fig. 6D) had significant increases with the Se dose of 0.250 mg plant^{-1} in BM737 and Nugrain420, and at Se dose of 0.500 mg plant^{-1} in Enforcer and Nugrain320.

In the field (Fig. 8C), at Se doses of 20 g ha^{-1} provided increments of 23.6 and 22.5% in relation to the control treatment and 10 g ha^{-1} of Se, respectively, in Nugrain320 in Lambari. Selenium dose of 40 g ha^{-1} increased the Zn content by 27.0% compared with 20 g ha^{-1} of Se, in Nugrain320 in Lavras. Zn contents in the shoot (Fig. 8D) were higher with Nugrain320. The main differences observed were the increments of 22.6% with 20 g ha^{-1} of Se in relation to the control treatment in Lambari and the increment of 20.7% with 40 g ha^{-1} in relation to 10 g ha^{-1} of Se in Lavras.

In the greenhouse, the highest Cu content in the grain occurred with 0.125 mg plant^{-1} of Se , which presented increments of 37.2, 25.0 and 29.7% in relation to the control

treatment, 0.250 and 0.500 mg plant^{-1} of Se, respectively, in BRS310. The dose of 0.125 mg plant^{-1} of Se also provided increments in Nugrain430 and SHS410. The dose of 0.250 mg plant-1 of Se was responsible for increments in Enforcer, K200, Nugrain320 and Nugrain420. And at doses of 0.500 mg plant^{-1} Se provided increments in BM737 (Fig. 7E). The dose of 0.500 mg plant^{-1} provided the highest Cu content in the shoot (Fig. 7F), which had increments of 22.71% compared with 0.250 mg plant^{-1}.

In the field, the Cu content in the grain (Fig. 8E) at a dose of 20 g ha^{-1} of Se provided the highest content, which was higher 17, 8 and 23.8% compared with the control treatment and 10 g ha^{-1} of Se, respectively, the Nugrain320 in Lambari. The dose of 40 g ha^{-1} of Se increased the contents by 20.6 and 36.9% compared with 10 and 20 g ha^{-1} of Se, respectively in Nugrain320 in Lavras. The Cu content in shoot (Fig. 8F) at the dose of 10 g ha^{-1} of Se was reduced by 22.1% compared with the control treatment at K200 in Lambari. Selenium doses had a similar effect on SHS410. However, the dose of 20 g ha^{-1} of Se provided increments in Nugrain430 in relation to the other applied doses. The doses of 10, 20 and 40 g ha^{-1} of Se provided increases of 24.3, 26.0 and 28.4% in relation to the control treatment in Enforcer in Lavras.

In the greenhouse, doses of 0.125 mg plant^{-1} of Se provided the highest Mn content in the grain in BRS310, which was 20.3 and 18.7% higher compared with the control treatment and 0.500 mg plant^{-1} of Se, respectively (Fig. 7G). In the field, the Mn content in the grain was not influenced by the applied Se doses. The lowest Mn content in shoots occurred with 10 g ha^{-1} of Se, which was 19.5, 13.8 and 15.9% lower than the control treatment, 20 and 40 g ha^{-1} of Se, respectively. on the BRS310 in Lambari. The dose of 10 g ha^{-1} of Se increased the content by 21.8% compared with the control treatment of BRS310 in Lavras. However, the 10 g ha^{-1} dose of Se decreased the content by 20.5% compared with the control treatment in Nugrain320 (Fig. 8G).

3.6. Principal Component Analysis and Pearson's Correlation

The principal component analysis provided a better understanding of the overall Se effect on the variables evaluated in this study. The first component (horizontal axis) represented most of the total variation in the three growing environments. The first two main components accounted for 50.82% of the total variation in the greenhouse (Fig. 9). The clusters were formed as a function of Se doses and cultivars. It is observed that in the greenhouse, the grain yield was influenced by Se doses of 0.125 and 0.250 mg plant^{-1}, with

emphasis on Nugrain430 concerning the other cultivars.

In the field-grown, Lambari (Fig. 10) and Lavras (Fig. 11), the response was 61.63% and 50.19%, respectively, of the total variation. It is observed that 20 and 40 g ha^{-1} of Se influenced grain yield. In Lavras Nugrain430 had higher grain yield, while in Lambari higher yield were obtained for BM737, BRS310, Enforcer and SHS410. Grain yield was grouped opposite to MnC-Gr, CuC-Gr, PC-Gr, PC-Sh, MgC-Gr, SC-Gr, ZnC-Gr, FeC-Gr, CaC-Gr in the three growing conditions. In the greenhouse, these variables mentioned above, CaC-Sh, APX, MgC-Sh, ZnC-Sh, NC-Gr, CAT, SC-Sh, CuC-Sh, NC-Sh, KC-Sh, Protein, and MDA also grouped opposite the grain yield. The response of SeC-Gr, SeC-Sh, SeU, SeAE, were a function of the applied doses of Se. Selenium dose of 40 g ha^{-1} on the opposite side of the control treatment was evident in the greenhouse and field Lavras. In Lambari, the overlapping groups indicate little influence of Se in doses of 10 and 20 g ha^{-1}.

Pearson's correlation for the response of plants in the greenhouse (Fig. 12) indicated that with an increase in grain yield there was an increase in FeC-Sh (r = 0.31), SeU (r = 0.25), SeAE (r = 0.28), H2O2 (r = 0.31), SeC-Gr (r = 0.01), SeC-Sh (r = 0.01). However, there was a decrease in APX (r = -0.25), MgC-Sh (r = -0.37), FeC- Gr (r = -0.23), CuC-Sh (r = -0.46), KC-Sh (r = -0.47), NC-Sh (r = -0.59), CaC-Gr (r = -0.49), CAT (r = -0.48), ZnC-Sh (r = -0.45), ZnC-Gr (r = -0.40), PC-Sh (r = -0 .65), SC-Gr (r = -0.52), MgC-Gr (r = -0.51), CuC-Gr (r = -0.55), PC-Gr (r = -0.59), NC-Gr (r = -0.72) and MnC-Gr (r = -0.65). The increase in CAT and APX enzymes implied an increase in ZnC-Sh, PC-Sh, SC-Gr, MgC-Gr, CuC-Gr, PC-Gr, NC-Gr and MnC-Gr with coefficients ranging from 0.06 to 0.56.

In the field-grown, Pearson’s correlation had a different response between Lambari and Lavras. In Lambari (Fig. 13) the increase in grain yield correlated positively with SeU (r = 0.33) and SeAE (r = 0.35). However, the increase in grain yield caused a decrease in the SC-Gr (r = -0.11), FeC-Gr (r = -0.26). The SeU (r = -0.03), SeC-Sh (r = -0.03) and SeAE (r = -0.11) correlated negatively with SC-Gr, causing a decrease. In Lavras (Fig.14) the increase in grain yield correlated positively, indicating increases in SeAE (r = 0.24), SeU (r = 0.31), SeC-Sh (r = 0.16), and SeC-Gr (r = 0.16). However, the increase in grain yield caused a reduction in, FeC-Sh (r = -0.39), CaC-Sh (r = -0.39), PC-Sh (r = -0.45), MnC-Sh (r = -0.33), FeC-Gr (r = -0.57), CuC-Gr (r = -0.32), NC-Gr (r = -0.68), ZnC-Gr (r = -0.38) and SC-Gr (r = -0.68). Selenium effects (SeAE, SeU, SeC-Sh and SeC-Gr) occurred mainly in relation to ZnC-Sh, FeC-Sh, SC-Gr, however, these correlations had coefficients smaller than |0.3|.

4. Discuss

The biochemical and physiological characteristics in which the Se acts are diverse, such as mitigating various abiotic stress (Lanza and Reis, 2021). Selenium promotes changes in antioxidant metabolism as a function of Se dose and Se source applied, cultivar and plant phenological stage. The response to different Se doses and cultivars can often be contradictory, as was the case in this study. Selenium application altered the metabolism of sorghum plants in some cultivars since it presented a reduction in MDA with the increase in the Se doses and lower H_2O_2 content with a higher Se dose in this study.

When at low concentrations, Se can prevent oxidative stress by increasing enzymatic and non-enzymatic activity, which makes the plants eliminate ROS due to the improvement of the defense system (Schiavon et al., 2017; Silva et al., 2018). However, other cultivars had observed increments in H_2O_2 and MDA contents that may have occurred because these plants reached a Se concentration that was phytotoxic, even though there were no visible phytotoxicity symptoms. According to Ríos et al. (2008), when Se is in concentration at the phytotoxicity level, it causes increases in the H_2O_2 content that influence the MDA.

With the reduction of H_2O_2 and MDA content, there was a more significant activity of antioxidant enzymes, superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) in some of the cultivars. However, the other cultivars had less expressive responses to the different Se doses. With the Se application, there is the formation of selenoproteins that act in the antioxidant metabolism, favoring the increase of the enzymatic activity (SOD, CAT and APX) that integrate the set that is responsible for the elimination of reactive oxygen species (ROS) and cellular detoxification (Lanza and Reis, 2021).

Selenium supply in increasing doses through sodium selenate via foliar was efficient in the sorghum biofortification. The positive effects of Se foliar biofortification have already been observed in wheat (Lara et al., 2019). Selenium content in the grain and the shoot increased as the Se doses increased. Other authors have also observed similar responses in rice and wheat (Delaqua et al., 2021; Lara et al., 2019; Lessa et al., 2020), even with differences in the number of applications and development stages.

Two Se applications were made in the present work, the first during flowering and the second during grain filling. In comparison, Lara et al. (2019) made two Se applications in the vegetative growth stage and the grain filling stage. Delaqua et al. (2021) made only one Se application in the vegetative growth phase. Lessa et al. (2020) made one Se application in the reproductive phase. Selenium content obtained may also be related to the fact that the

surfactant was added to the Se solution since the surfactant promotes a better contact between the applied solution and the leave (Knijnenburg et al., 2018), as already observed in studies with wheat from Lara et al. (2019). When surfactants are not used, there may be less absorption of the applied nutrient solution due to the dripping of the drops when in contact with the leaf surface.

Although there was an increase in the Se accumulation in the grain as a function of the Se dose to all cultivars, the Se maximum limit must be considered. With the application of foliar solutions, the droplets are directed to all leaves in greenhouse-grown plants. Thus, in the field-grown foliar sprays, lower Se recovery rates can be expected (Ramkisson et al., 2019). This difference in Se doses is because, in the greenhouse, the foliar application has a better improvement than in field conditions. In the field, the plants are closer, with an overlap in the leaves during foliar application.

The genetic characteristics of the cultivar that can influence better use of Se applied, since absorption and translocation are independent processes, it is relevant to select cultivars that, in addition to Se absorbing, also translocate this element to the edible plants part (Silva et al., 2021), in this case to sorghum grains. Studies with this objective were carried out in other cultures to select genotypes responsive to Se biofortification in cowpea (Silva et al., 2021) and rice (Zhang et al., 2019).

The higher grain yield and grain weight observed in some cultivars may be related to beneficial effects that Se provides to plants at low concentrations, improving growth, quality, and grain yield (Subramanyam et al., 2019). Selenium affects enzymes such as catalase (CAT) and superoxide dismutase (SOD), causing an anti-senescence effect and an improvement in the antioxidant defense system, which may explain the observed benefits in grain yield (Hasanuzzaman et al., 2020).

In addition to the effects on grain yield, it is essential to know how Se interferes with the absorption of macronutrients and micronutrients in a biofortification program. Assessment of nutrient concentration in the shoot is the common result covering dry matter accumulation and mineral nutrient uptake (Jarrell and Beverly, 1981). The lowest nutrient content and the highest grain yield in field cultivation occurred in sorghum cultivated in Lavras when compared with Lambari. This difference observed in plants in these environments is probably due to the dilution effect. This inverse relationship between growth and mineral concentration occurs when dry weight accumulation increases faster than nutrient accumulation (Jarrell and Beverly, 1981) which characterizes the dilution effect.

Mineral nutrients and their remobilization efficiency vary with plant species (Maillard et al., 2015), and the uptake and distribution of macronutrients by plants are affected by plant development (Moreira and Fageria, 2009). Nutrient content and remobilization in plants are generally related to leaf senescence, allowing young plant tissues to obtain nutrients and contribute to nutrient use efficiency (Abdallah et al., 2010; Fischer, 2007). Remobilization takes place mainly through the phloem. According to White (2012), it is known that N, P, S, K and Mg, except for Ca, have high mobility in the phloem. While Fe, Zn, Cu, Ni, Mo, B and Cl, except for Mn, show moderate mobility.

Selenium and S have chemical similarities, so S transporters and enzymes are the primary means of Se absorption and assimilation, affecting the N assimilation pathway (Schiavon et al., 2016). Selenium effects on the metabolic pathways of S, N and phenol can be defined by the cultivation method, Se dose, selenate/sulfate and time of exposure to Se fertilization (Schiavon et al., 2016). According to Golubkina et al. (2018), the Se application affects the N metabolism, protein and amino acid biosynthesis and, in particular, the amino acid phenylalanine, which is a precursor of phenolic compounds like flavonoids.

Since the macronutrients were supplied through fertilization in adequate amounts for the growth and development of sorghum plants, they may have favored the interaction between Se and the macronutrients. Selenium, S and N interaction was observed. This interaction may have occurred because N activates the S metabolism, promoting S assimilation and, consequently, increases in O-acetyl serine, cysteine and proteins (Kim et al., 1999). The fact that S and Se use the same metabolic pathway indirectly favors the Se absorption by plants, being metabolized into selenoproteins (Zhou et al., 2020). The S, P and K absorption, and other mineral elements such as Se, are also favored by N, as this nutrient promotes plant growth (Chen et al., 2012).

Phosphorus, K, Mg and Fe are abundant in sorghum (Pontieri et al., 2014). The effects observed with Se foliar application in sorghum are similar to the results observed by Hawrylak-Nowak (2008) in maize. This author observed that a function of the applied Se dose might not change the K content, as it may cause reductions or increases. Selenium slightly influenced the Mg contents in sorghum. In studies with maize, there was no effect on Mg contents when using Se through selenate (Hawrylak-Nowak, 2008). This author also observed increases in the Ca content in the shoot concerning the control in maize plants. In this work, we found increments in grain and shoot of sorghum plants.

In general, it was found that Se supply through selenate altered Fe, Zn, Cu and Mn

contents in sorghum plants. Selenium supply may have changed the ionic permeability coefficient in the plasma membrane, thus limiting the transport and accumulation of micronutrients in plant cells. Cu increments in the biomass of *Catharanthus roseus* (L.) were observed by Arvy et al. (1995) when Se was applied. However, Schiavon et al. (2013) reported in the shoot of tomato plant (*Solanum lycopersicon* L.). As Cu, Fe, Zn, and Mn are cofactors of SOD (Bocchini et al., 2018), the changes observed in these elements may be related to the activity of this enzyme.

Possible synergisms or antagonisms between Se and micronutrients may be related to the Se source used, e.g., selenate. The increase in Fe, Zn and Cu contents in the grains may indicate a possible synergistic effect of the selenate with these elements. Boldrin et al. (2013) observed a synergistic effect due to increases in the Cu, Zn and Mn contents in the rice grain with the Se application. Mineral content is highly correlated with protein content because it involves protein biosynthesis (Cramer et al., 2011). Furthermore, during the maturation of cereal plants, Fe, Zn, Mn and proteins are transferred from leaves to seeds (Brinch-Pedersen et al., 2007).

5. Conclusion

All cultivars responded positively to Se foliar fertilization through sodium selenate in the different growing conditions under study. However, there were different responses among the assessed cultivars after Se addition. Selenium fertilization increased the grain yield of the evaluated cultivars. With the highest grain yield observed in Nugrain420 and Nugrain430 in a greenhouse with 0.500 mg plant^{-1} of Se. In the field, Lavras and Lambari, the highest grain yield occurred with Nugrain430 and Nugrain320 with 20 and 40 g ha^{-1} of Se respectively. Selenium application affected the antioxidant metabolism and mineral content of the studied cultivars. Selenium efficiency absorption in K200 in the field (Lavras and Lambari), it was higher with 20 g ha^{-1} of Se. In the grain, the main Se effects concerning other nutrients were observed under greenhouse cultivation. The synergisms occurred between Se and K, Fe, Zn, Cu, and N. The antagonism occurred between Se and S, Ca, Mg, P and Mn. Therefore, sorghum plants respond positively to the Se foliar application, and the ingestion of sorghum grains Se biofortified can provide Se for humans and animals.

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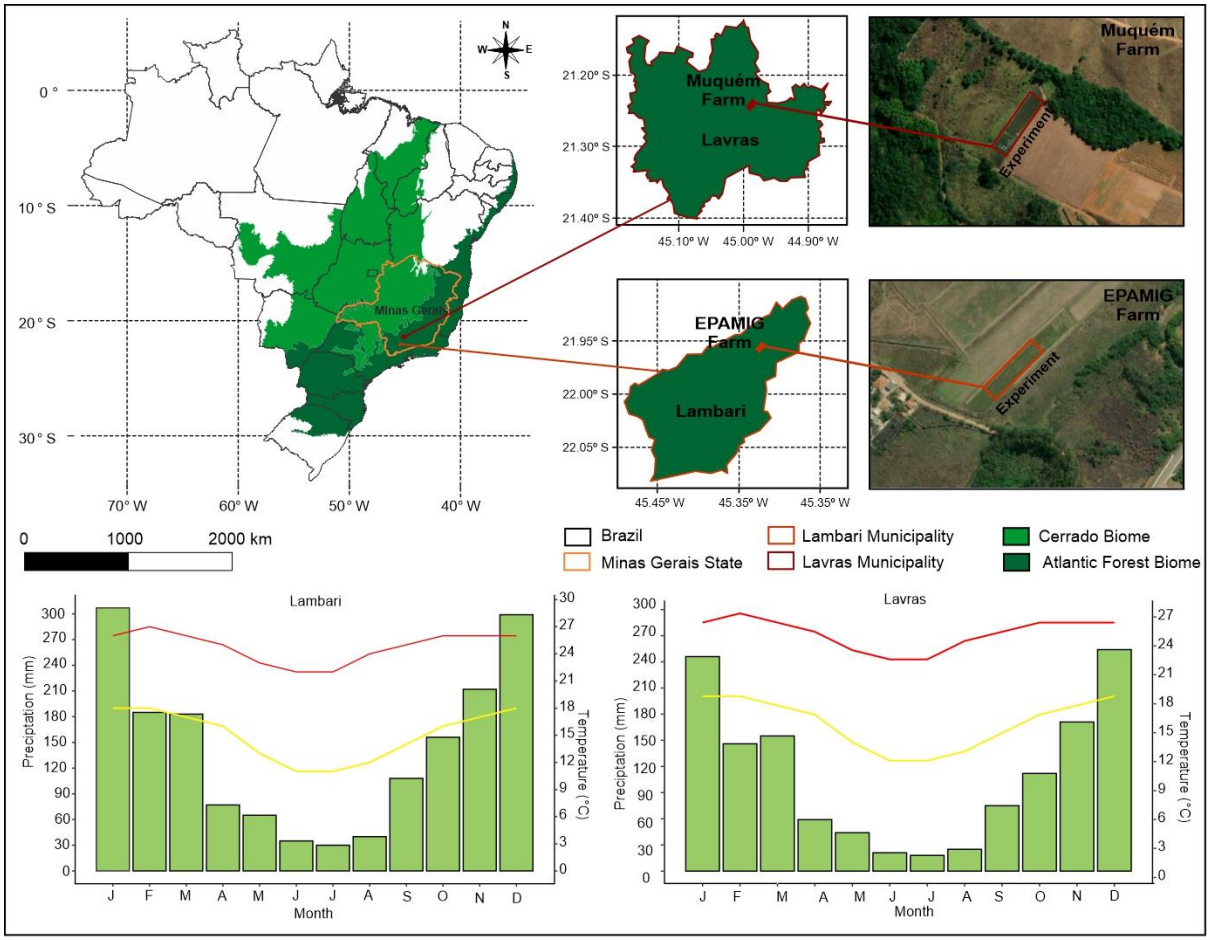

Fig. 1 Location of the experimental area and precipitation, minimum and maximum temperature: based on climatological averages calculated from a 30-year observed data series. Source: Climatempo.

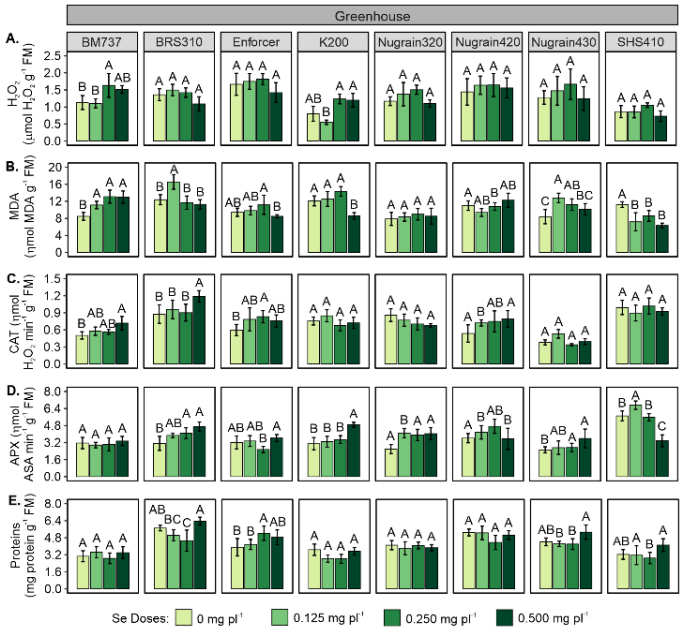

Fig. 2 Greenhouse-grown conditions. Hydrogen peroxide – H_2O_2 (A), lipid peroxidation by the content of malondialdehyde - MDA (B), catalase – CAT (C), ascorbate peroxidase – APX (D), and proteins in extract enzymatic (E). Different letters indicate significant differences between treatments at a probability level of 5% ($p<0.05$) by test Tukey. The bars show means, and the vertical error bars refer to the standard errors (n=4).

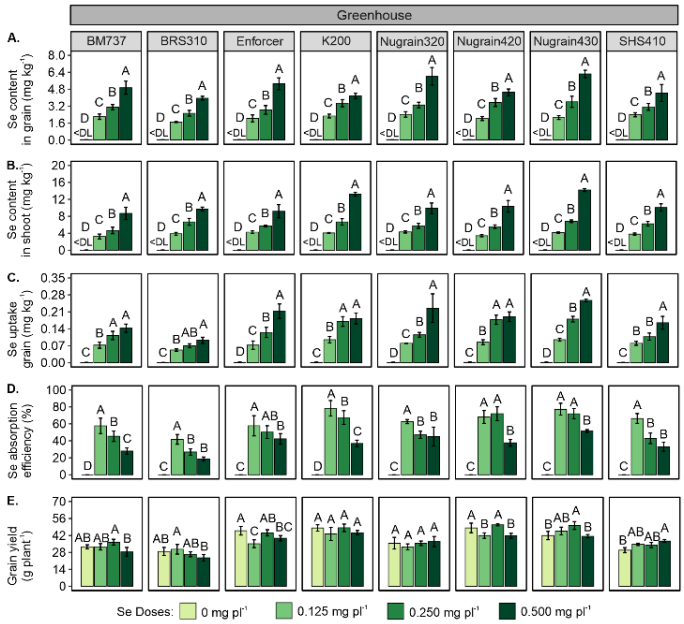

Fig. 3 Greenhouse-grown conditions. Selenium content in grain (A), Se content in the shoot (B), Se uptake by grain (C), Se absorption efficiency (D) and grain yield (E). Different letters indicate significant differences between treatments at a probability level of 5% ($p<0.05$) by test Tukey. The bars show means, and the vertical error bars refer to the standard errors (n=4).

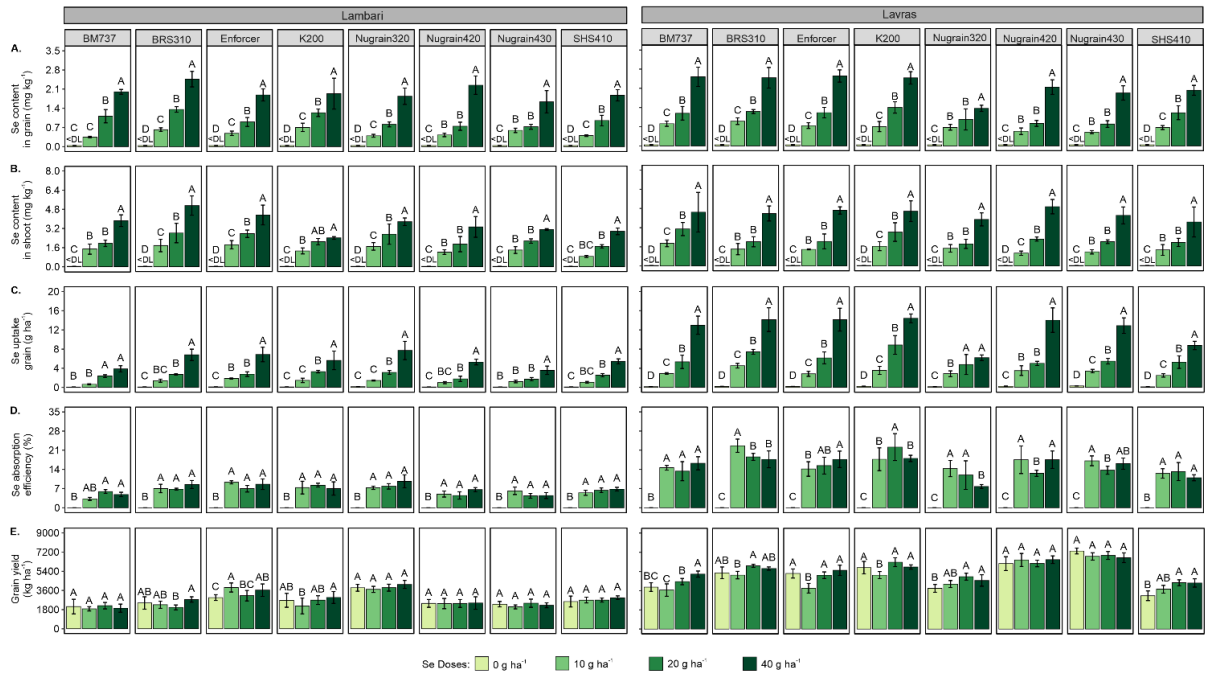

Fig. 4 Field-grown conditions. Selenium content in grain (A), Se content in the shoot (B), Se uptake by grain (C), Se absorption efficiency (D) and grain yield (E). Different letters indicate significant differences between treatments at a probability level of 5% ($p<0.05$) by test Tukey. The bars show means, and the vertical error bars refer to the standard errors (n=4).

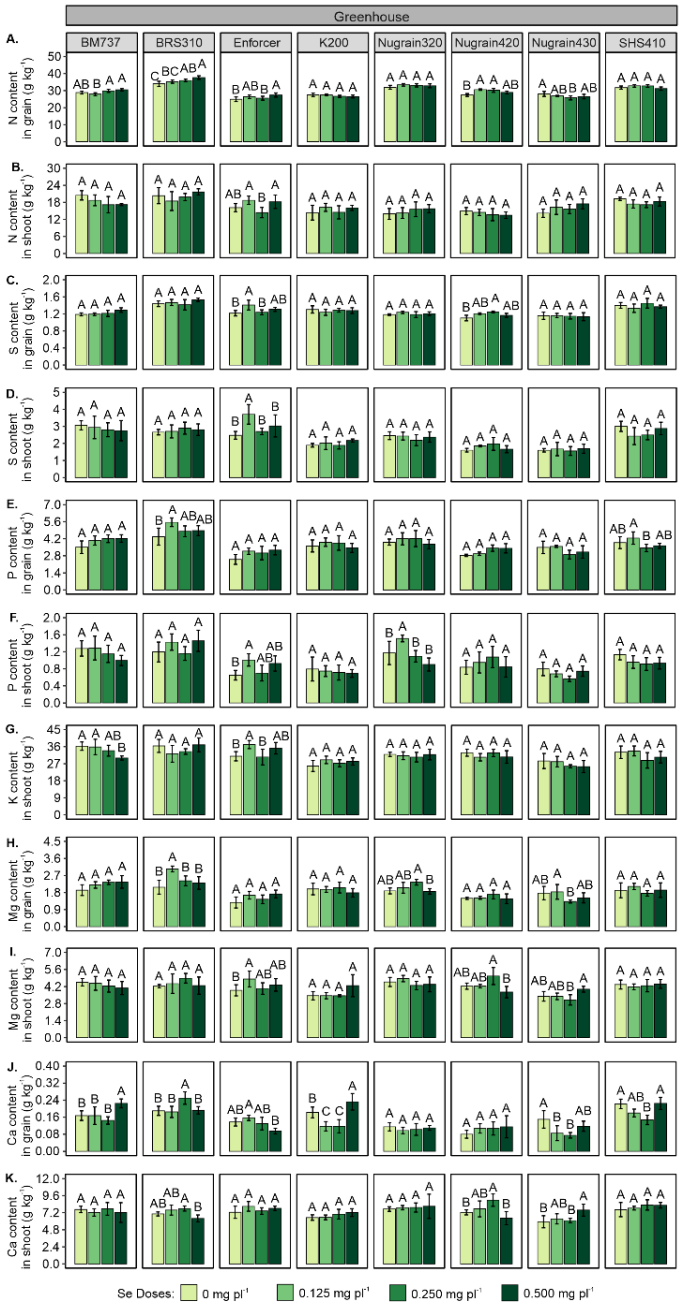

Fig. 5 Greenhouse-grown conditions. The macronutrients contents in the grain and the shoot of the sorghum plants. Different letters indicate significant differences between treatments at a probability level of 5% ($p<0.05$) by test Tukey. The bars show means, and the vertical error bars refer to the standard errors (n=4).

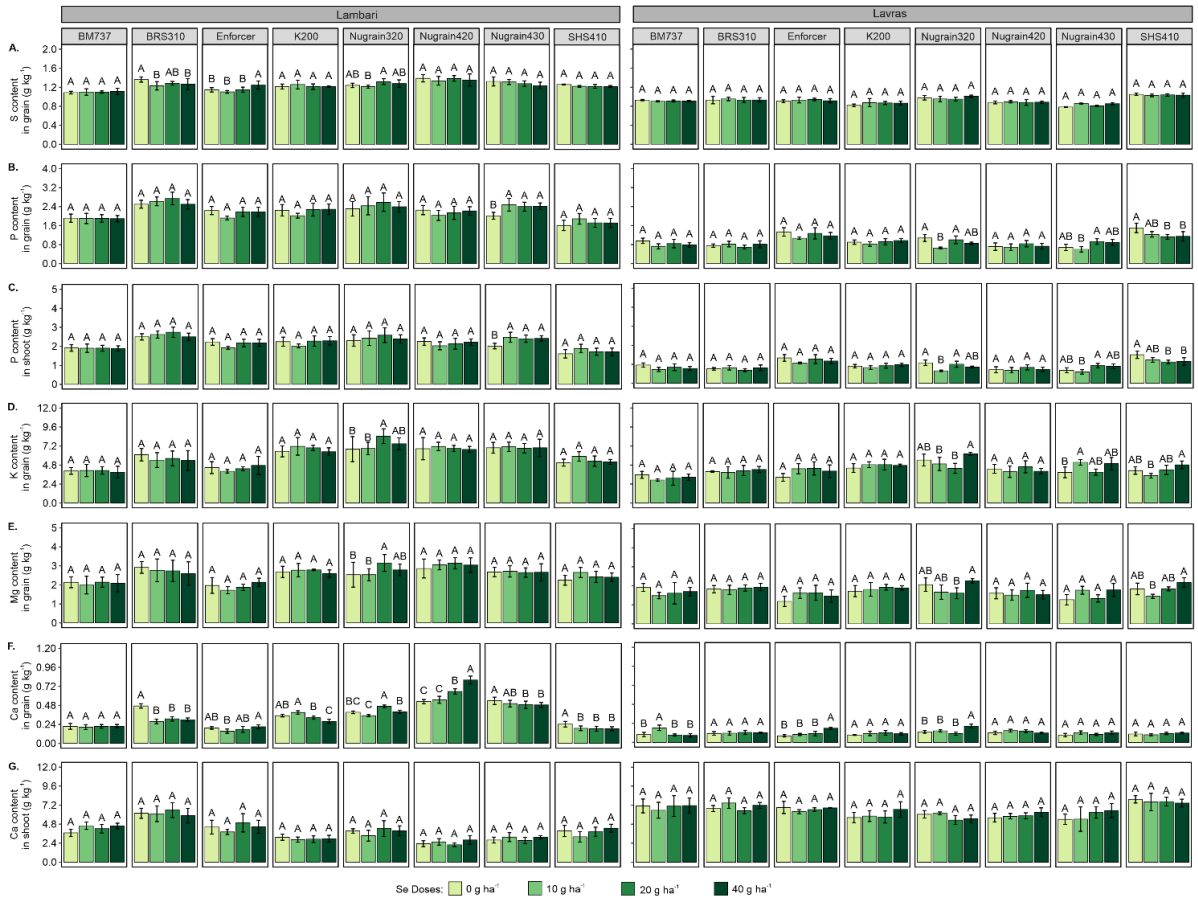

Fig. 6 Field-grown conditions. The macronutrients contents in the grain and the shoot of the sorghum plants. Different letters indicate significant differences between treatments at a probability level of 5% ($p<0.05$) by test Tukey. The bars show means, and the vertical error bars refer to the standard errors ($n=4$).

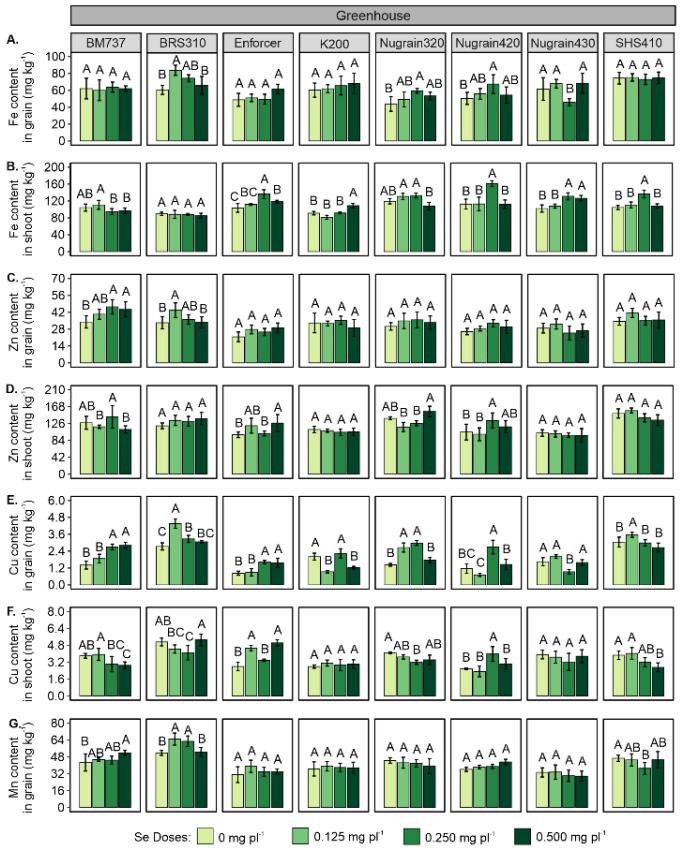

Fig. 7 Greenhouse-grown conditions. The micronutrients contents in the grain and the shoot of the sorghum plants. Different letters indicate significant differences between treatments at a probability level of 5% ($p<0.05$) by test Tukey. The bars show means, and the vertical error bars refer to the standard errors (n=4).

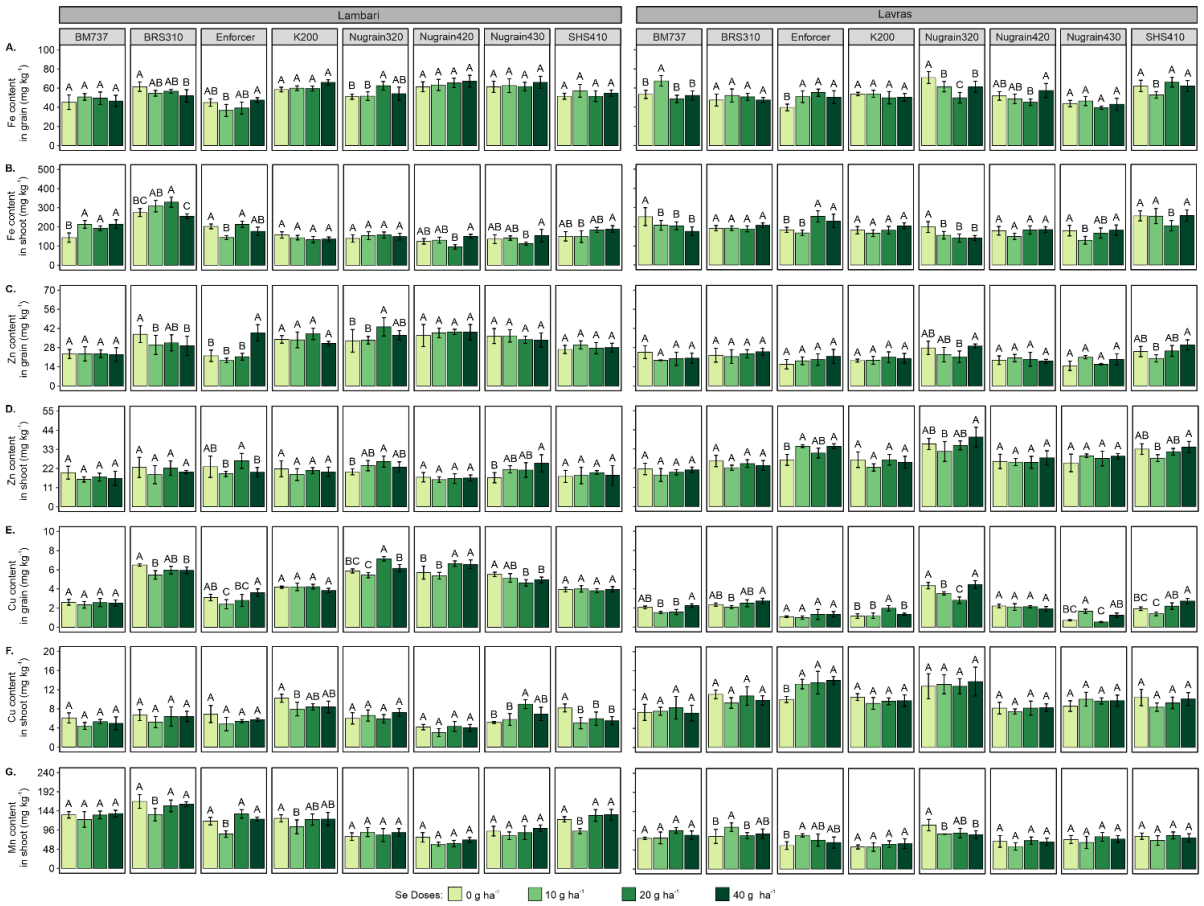

Fig. 8 Field-grown conditions. The micronutrients contents in the grain and the shoot of the sorghum plants. Different letters indicate significant differences between treatments at a probability level of 5% ($p<0.05$) by test Tukey. The bars show means, and the vertical error bars refer to the standard errors (n=4).

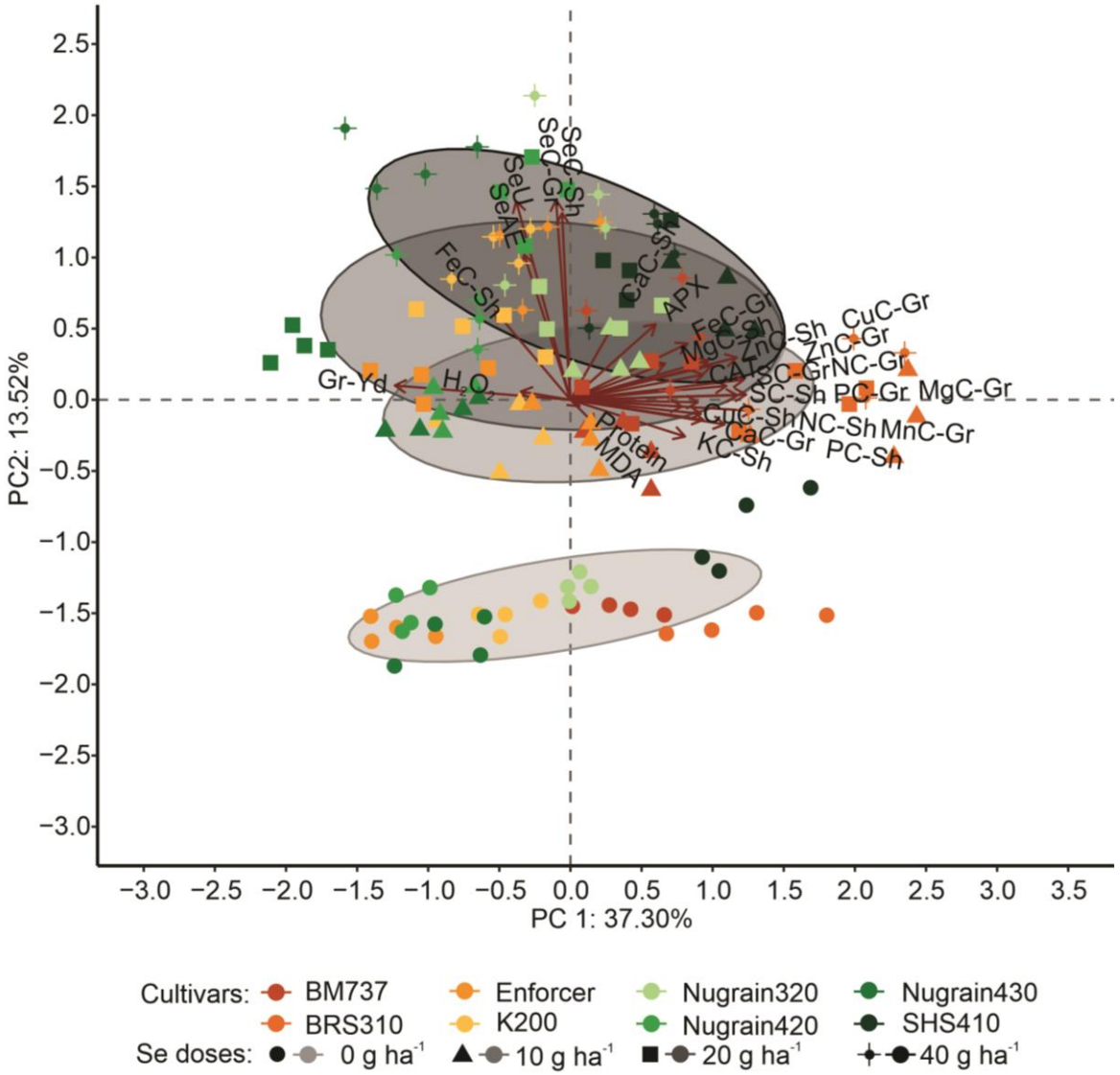

Fig. 9 Principal component in the greenhouse-grown. Abbreviations: Se content in grain (SeC-Gr) and in shoot (SeC-Sh); Se absorption efficiency (SeAE); Se uptake (SeU); lipid peroxidation (MDA); hydrogen peroxide (H_2O_2); catalase (CAT); ascorbate peroxidase (APX); grain yield (Gr-Yd); N (NC-Gr), S (SC-Gr), P (PC-Gr), K (KC-Gr), Mg (MgC-Gr), Fe (FeC-Gr), Zn (ZnC-Gr), Mn (MnC-Gr), Cu (CuC-Gr) content in the grain; N (NC-Sh), S (SC-Sh), P (PC-Sh), K (KC-Sh), Mg (MgC-Sh), Ca (CaC-Sh); Fe (FeC-Sh); Zn (ZnC-Sh), Mn (MnC-Sh) and Cu (CuC-Sh) content in the shoot.

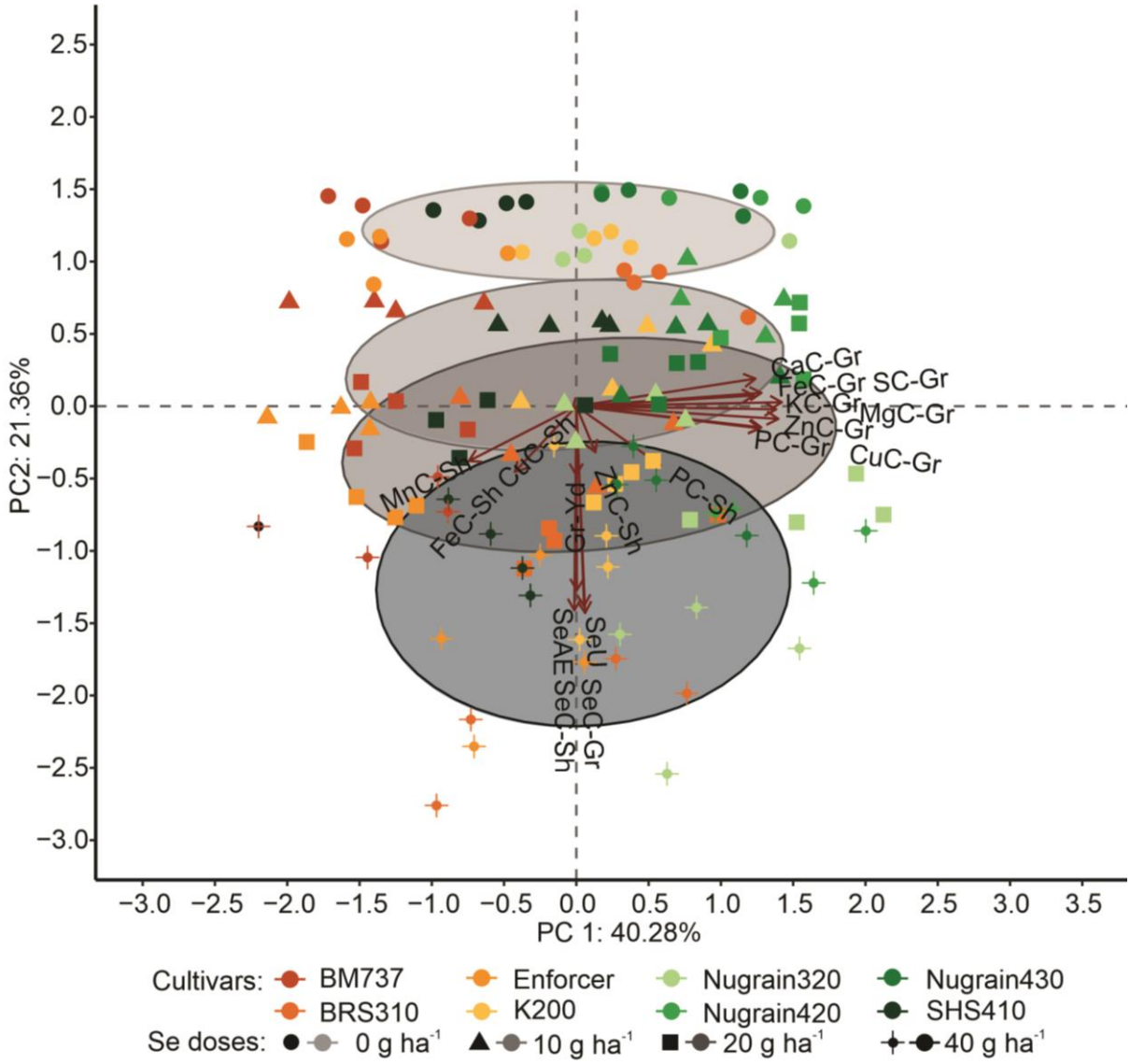

Fig. 10 Principal component analysis field-grown sorghum at Lambari Abbreviations: Se content in grain (SeC-Gr) and in shoot (SeC-Sh); Se absorption efficiency (SeAE); Se uptake (SeU); grain yield (Gr-Yd); N (NC-Gr), S (SC-Gr), P (PC-Gr), K (KC-Gr), Mg (MgC-Gr), Fe (FeC-Gr), Zn (ZnC-Gr), Mn (MnC-Gr), Cu (CuC-Gr) content in the grain; N (NC-Sh), S (SC-Sh), P (PC-Sh), K (KC-Sh), Mg (MgC-Sh), Ca (CaC-Sh); Fe (FeC-Sh); Zn (ZnC-Sh), Mn (MnC-Sh) and Cu (CuC-Sh) content in the shoot.

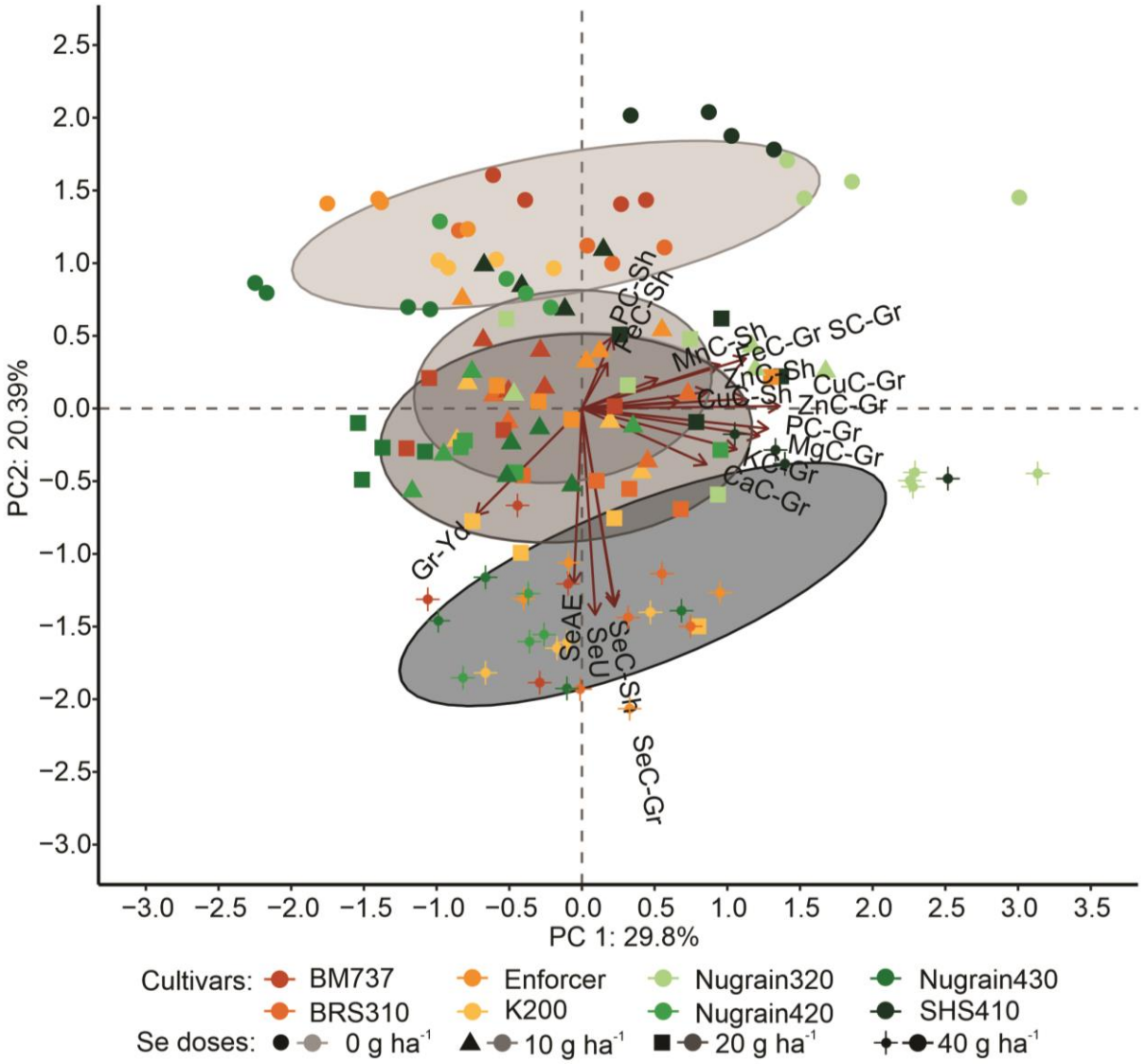

Fig. 11 Principal component analysis field-grown sorghum at Lavras. Abbreviations: Se content in grain (SeC-Gr) and in shoot (SeC-Sh); Se absorption efficiency (SeAE); Se uptake (SeU); grain yield (Gr-Yd); N (NC-Gr), S (SC-Gr), P (PC-Gr), K (KC-Gr), Mg (MgC-Gr), Fe (FeC-Gr), Zn (ZnC-Gr), Mn (MnC-Gr), Cu (CuC-Gr) content in the grain; N (NC-Sh), S (SC-Sh), P (PC-Sh), K (KC-Sh), Mg (MgC-Sh), Ca (CaC-Sh); Fe (FeC-Sh); Zn (ZnC-Sh), Mn (MnC-Sh) and Cu (CuC-Sh) content in the shoot.

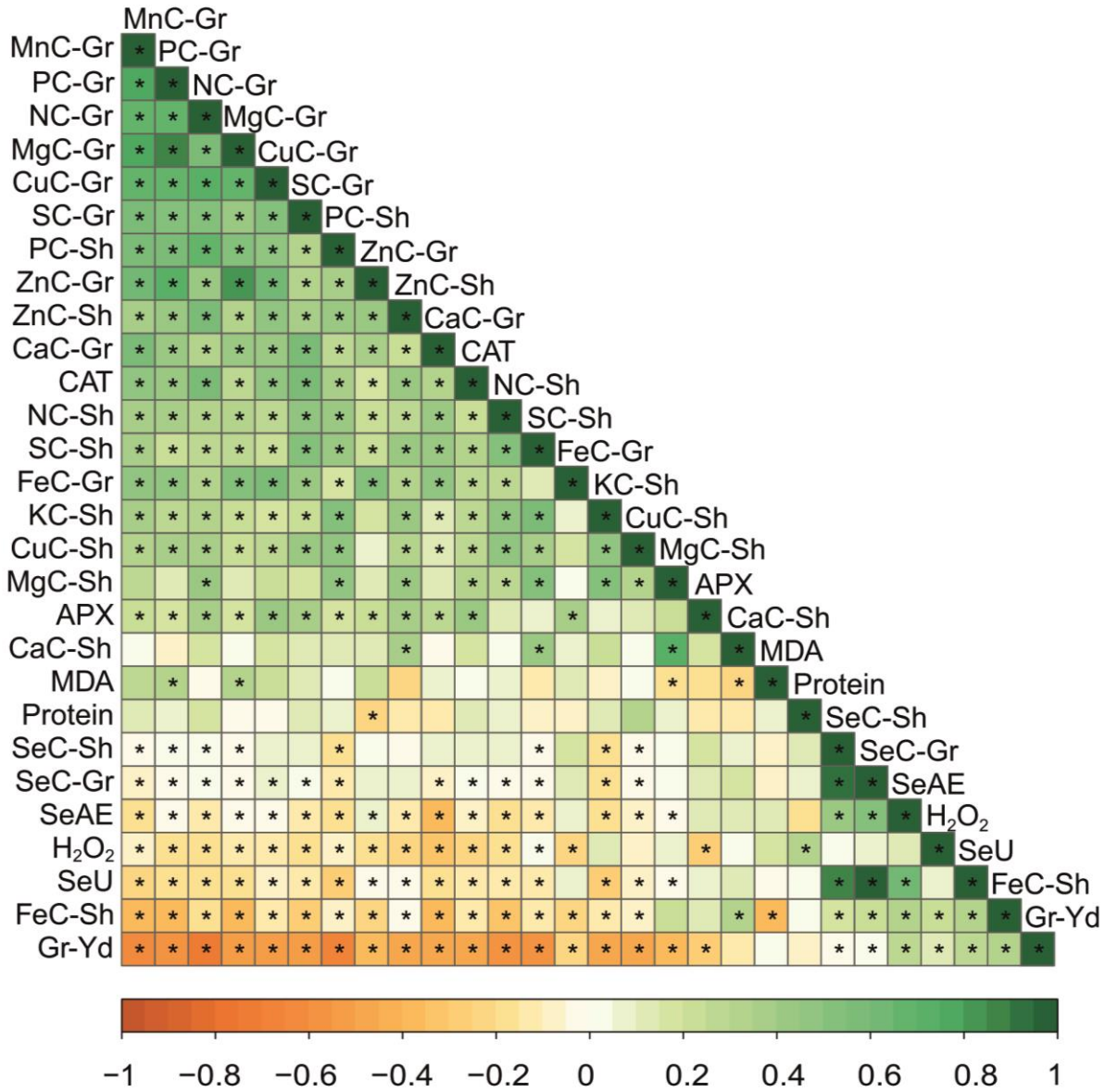

Fig. 12 Heatmap showing the Pearson's correlation the significance of relationship identified with * was significant by F-test at $p \leq 0.05$ in the greenhouse-grown. Abbreviations: Se content in grain (SeC-Gr) and in shoot (SeC-Sh); Se absorption efficiency (SeAE); Se uptake (SeU); lipid peroxidation (MDA); hydrogen peroxide (H_2O_2); catalase (CAT); ascorbate peroxidase (APX); grain yield (Gr-Yd); N (NC-Gr), S (SC-Gr), P (PC-Gr), K (KC-Gr), Mg (MgC-Gr), Fe (FeC-Gr), Zn (ZnC-Gr), Mn (MnC-Gr), Cu (CuC-Gr) content in the grain; N (NC-Sh), S (SC-Sh), P (PC-Sh), K (KC-Sh), Mg (MgC-Sh), Ca (CaC-Sh); Fe (FeC-Sh); Zn (ZnC-Sh), Mn (MnC-Sh) and Cu (CuC-Sh) content in the shoot.

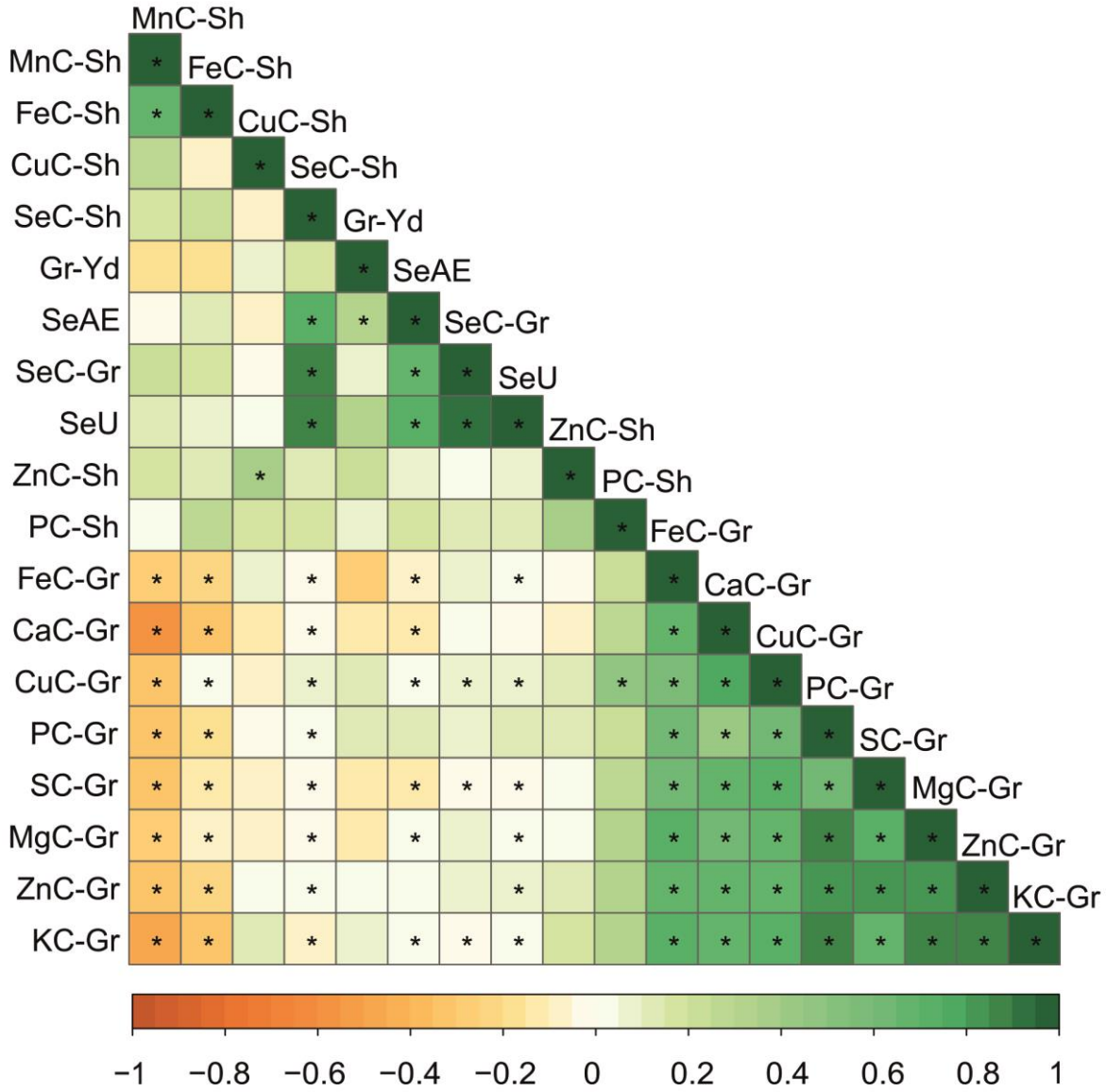

Fig. 13 Heatmap showing the Pearson's correlation the significance of relationship identified with * was significant by F-test at $p \leq 0.05$ in the field-grown at Lambari. Abbreviations: Se content in grain (SeC-Gr) and in shoot (SeC-Sh); Se absorption efficiency (SeAE); Se uptake (SeU); grain yield (Gr-Yd); N (NC-Gr), S (SC-Gr), P (PC-Gr), K (KC-Gr), Mg (MgC-Gr), Fe (FeC-Gr), Zn (ZnC-Gr), Mn (MnC-Gr), Cu (CuC-Gr) content in the grain; N (NC-Sh), S (SC-Sh), P (PC-Sh), K (KC-Sh), Mg (MgC-Sh), Ca (CaC-Sh); Fe (FeC-Sh); Zn (ZnC-Sh), Mn (MnC-Sh) and Cu (CuC-Sh) content in the shoot.

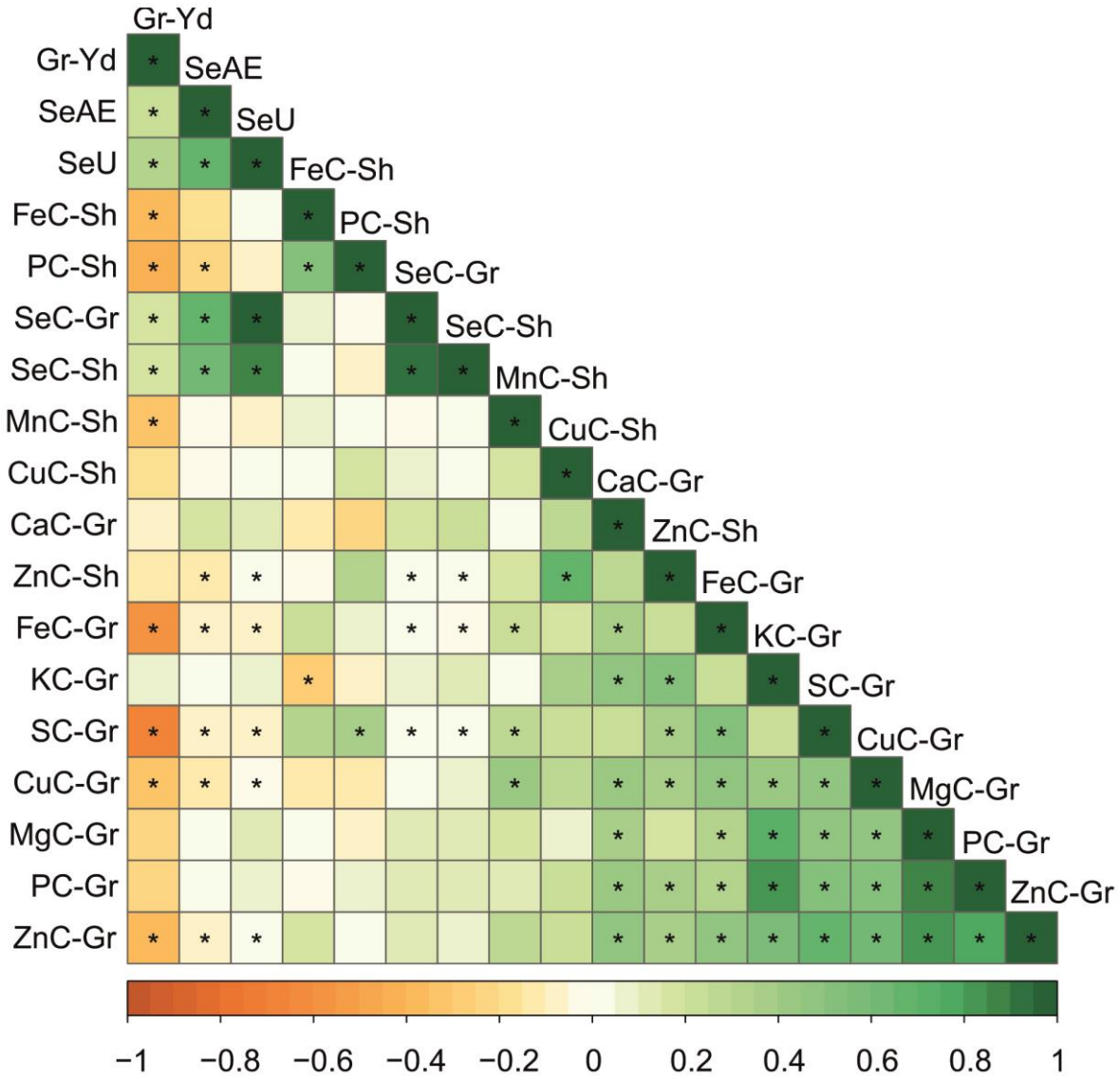

Fig. 14 Heatmap showing the Pearson's correlation the significance of relationship identified with * was significant by F-test at $p \leq 0.05$ in the field-grown sorghum at Lavras. Abbreviations: Se content in grain (SeC-Gr) and in shoot (SeC-Sh); Se absorption efficiency (SeAE); Se uptake (SeU); grain yield (Gr-Yd); N (NC-Gr), S (SC-Gr), P (PC-Gr), K (KC-Gr), Mg (MgC-Gr), Fe (FeC-Gr), Zn (ZnC-Gr), Mn (MnC-Gr), Cu (CuC-Gr) content in the grain; N (NC-Sh), S (SC-Sh), P (PC-Sh), K (KC-Sh), Mg (MgC-Sh), Ca (CaC-Sh); Fe (FeC-Sh); Zn (ZnC-Sh), Mn (MnC-Sh) and Cu (CuC-Sh) content in the shoot.

Material Supplementary

Table S1 Chemical characterization and particle size distribution before sowing of the soil used in greenhouse-grown and field-grown sorghum in Lambari and Lavras

	Unit	Method/ Extractant	Greenhouse	Lambari		Lavras	
				0-20cm	20-40cm	0-20cm	20-40cm
Sand	g kg^{-1}	Hydrometer	730	250	440	570	520
Silt	g kg^{-1}	Hydrometer	50	200	260	160	180
Clay	g kg^{-1}	Hydrometer	220	550	300	270	300
SOM	g kg^{-1}	$Na_2Cr_2O_7$ 4N + H_2SO_4 10N	6.8	4.82	4.08	2.04	1.54
pH		Water 1:2.5	5.1	5.7	5.6	5.8	6.0
Al	$cmol_c$ dm^{-3}	KCl (1 mol L^{-1})	0.45	0.18	0.24	0.14	0.09
Al+H	$cmol_c$ dm^{-3}	SMP	2.37	5.83	6.38	1.96	2.48
P	mg dm^{-3}	Mehlich-1	0.71	58.32	36.16	5.60	1.72
K	mg dm^{-3}	Mehlich-1	23.46	115.2	77.7	57.8	43.3
Ca	$cmol_c$ dm^{-3}	KCl (1 mol L^{-1})	0.19	4.84	2.53	3.12	2.50
Mg	$cmol_c$ dm^{-3}	KCl (1 mol L^{-1})	0.10	1.70	0.96	0.85	0.77
S	mg dm^{-3}	$Ca(H_2PO_4)_2$ + CH_3COOH	4.23	9.63	18.97	3.57	3.49
B	mg dm^{-3}	Hot water	0.07	0.14	0.10	0.22	0.12
Cu	mg dm^{-3}	Mehlich-1	0.10	1.88	1.81	1.06	0.76
Fe	mg dm^{-3}	Mehlich-1	47.33	32.19	23.73	61.86	45.11
Mn	mg dm^{-3}	Mehlich-1	2.44	8.88	5.20	30.39	20.33
Zn	mg dm^{-3}	Mehlich-1	0.28	0.85	0.54	1.76	0.70
Se	mg kg^{-1}	USEPA3051A	<DL	<DL	<DL	<DL	<DL

SOM – soil organic matter; DL – detect limited

Table S2 Lines used to determine the elements with ICP-OES and assessment of precision through the analysis of Tomato Leaves SRM1573a

Element or Nutrient	λ (nm)	Certified[a] (mg kg^{-1})	Found[b] (mg kg^{-1})	% Recovery[c]
S	180.731	9600	8937±292.1	93
P	178.287	2161±26	2040±46.4	94
K	769.896	26760±480	29546±745.7	110
Ca	315.887	50450 ±550	49700±1059	99
Mg	279.079	1200	11276±274.7	94
Fe	373.486	367.5 ±4.3	360.97±18.45	98
Cu	324.754	4.70±0.14	3.61±0.29	77
Zn	213.856	30.94±0.55	27.28±0.85	88
Mn	403.076	246.3±7.1	270.80±6.89	110

[a] Results for Tomato Leaves SRM1573a represented as mean ± confidence interval, informative value.
[b] Mean ± standard error of the mean. Average of ten determinations. [c] Average of ten determinations

Table S3 Summary of characteristics of eight sorghum cultivars

Cultivars	RNC*	PH (cm)	SF (days)	SH (days)	PS	PD	Grain color
BM737	29344	135-145	51-55	120-130	elliptic	semi-compact	red
BRS310	18751	115	65	120	partially open	UN	red
Enforcer	30336	120-130	55-60	110-120	elliptic	semi-compact	brown
K200	38142	140-160	UN	UN	elliptic	semi-compact	red
Nugrain320	38145	130	58-60	110-115	elliptic	semi-compact	orange
Nugrain420	40315	150	65-70	125-130	elliptic	partially open	orange
Nugrain430	36743	140	65-70	125-130	elliptic	semi-compact	orange
SHS-410	18772	125-135	48-52	120-130	UN	UN	red

UN - uninformed; PH - plant height; SF – sowing to flowering; SH – sowing to harvest; PS – panicle shape; PD – panicle density; *RNC- National Registry of cultivars

Table S4 - Analysis of variance of 8 sorghum cultivars cultivated in greenhouse and in field conditions, fertilized with different Se doses.

Source of variation	Greenhouse				Field - Lambari					Field Lavras				
	C	D	CxD	CV	B	C	D	CxD	CV	B	C	D	CxD	CV
H_2O_2	**	**	*	18.73	---	---	---	---	---	---	---	---	---	---
MDA	**	**	**	12.56	---	---	---	---	---	---	---	---	---	---
SOD	**	ns	ns	12.38	---	---	---	---	---	---	---	---	---	---
CAT	**	*	**	15.32	---	---	---	---	---	---	---	---	---	---
APX	**	**	**	13.90	---	---	---	---	---	---	---	---	---	---
Protein	**	**	**	13.12	---	---	---	---	---	---	---	---	---	---
SeC-Gr	**	**	**	13.28	ns	**	**	**	21.54	**	**	**	**	16.98
SeC-Sh	**	**	**	12.31	ns	**	**	**	22.53	ns	*	**	ns	27.16
SeU	**	**	**	17.00	ns	**	**	**	28.52	**	**	**	**	20.27
SeAE	**	**	**	15.05	ns	**	**	**	22.31	*	**	**	**	20.55
Grain yield	**	**	**	7.62	**	**	*	*	13.57	*	**	**	**	7.52
Grain weight	**	ns	ns	15.18	**	**	**	ns	8.00	ns	**	*	ns	5.27
NC-Gr	**	**	**	3.27	*	**	ns	ns	5.25	ns	**	ns	ns	4.97
KC-Gr	**	ns	ns	14.64	ns	**	ns	ns	13.16	ns	**	*	**	13.67
PC-Gr	**	*	*	11.18	ns	**	ns	ns	13.49	ns	**	*	**	16.75
CaC-Gr	**	**	**	17.77	*	**	**	**	8.22	ns	**	**	**	16.68
MgC-Gr	**	**	**	13.15	ns	**	ns	ns	14.39	ns	**	*	**	16.13
SC-Gr	**	ns	**	5.28	**	**	ns	ns	4.91	ns	**	ns	ns	4.20
FeC-Gr	**	*	**	12.96	ns	**	ns	**	8.96	**	**	*	**	8.88
CuC-Gr	**	**	**	12.14	ns	**	**	**	7.78	**	**	**	**	12.01
Mn C-Gr	**	ns	**	11.69	*	**	ns	ns	15.01	ns	**	ns	ns	15.39
Zn C-Gr	**	**	*	14.67	*	**	ns	**	14.59	ns	**	*	*	17.05
NC-Sh	**	ns	*	11.09	**	**	*	ns	13.43	ns	**	ns	*	15.08
KC-Sh	**	*	**	8.96	ns	**	ns	ns	12.37	ns	**	ns	*	9.23
PC-Sh	**	**	**	18.39	*	**	ns	ns	9.62	*	**	**	**	13.61
CaC-Sh	**	*	**	9.86	**	**	ns	ns	14.77	ns	**	ns	ns	10.70
MgC-Sh	**	ns	**	11.16	**	**	ns	ns	13.53	ns	**	ns	ns	12.06
SC-Sh	**	ns	*	14.49	**	**	**	ns	10.28	ns	**	ns	ns	11.78
FeC-Sh	**	**	**	6.95	ns	**	*	**	10.85	ns	**	**	**	12.38
CuC-Sh	**	*	**	12.65	**	**	**	**	16.44	**	**	ns	*	12.43
MnC-Sh	**	ns	ns	11.77	ns	**	**	**	10.80	*	**	ns	**	13.51
ZnC-Sh	**	ns	**	10.66	**	**	*	*	16.39	**	**	**	**	10.26

^{ns} – not significant by F-test; * - significant by F-test at p<0,05; ** - significant by F-test at p<0,01; CV – coefficient of variation (%). Degrees of freedom, greenhouse: cultivars (C) – 7; doses (D) – 3; C x D – 21; residue – 96. Degrees of freedom, field: block (B) – 3; cultivars (C) – 7; doses (D) – 3; C x D – 21; residue – 93.

Abbreviations: Se content in grain (SeC-Gr) and in shoot (SeC-Sh); Se absorption efficiency (SeAE); Se uptake (SeU); lipid peroxidation (MDA); hydrogen peroxide (H_2O_2); catalase (CAT); ascorbate peroxidase (APX), superoxide dismutase (SOD), proteins in extract enzymatic (Prot); N (NC-Gr), S (SC-Gr), P (PC-Gr), K (KC-Gr), Mg (MgC-Gr), Fe (FeC-Gr), Zn (ZnC-Gr), Mn (MnC-Gr), Cu (CuC-Gr) content in the grain; N (NC-Sh), S (SC-Sh), P (PC-Sh), K (KC-Sh), Mg (MgC-Sh), Ca (CaC-Sh); Fe (FeC-Sh); Zn (ZnC-Sh), Mn (MnC-Sh) and Cu (CuC-Sh) content in the shoot.

Table S5 Joint-variance analysis of eight sorghum cultivars in field-grown conditions (Lambari and Lavras) fertilized with different Se doses

Source of variation	FMax	F-test									CV (%)
		Block (B)	Cultivars (C)	Doses (D)	Local (L)	BxL	CxD	LxC	LxD	LxCxD	
SeC-Gr	1.216	ns	**	**	**	*	**	**	**	**	19.13
SeC-Sh	1.747	ns	**	**	**	ns	**	**	**	**	25.18
SeU	2.600	*	**	**	**	**	**	**	**	**	23.40
SeAE	4.708	*	**	**	**	**	**	**	**	**	22.46
Grain yield	1.154	ns	**	**	**	**	**	**	**	**	9.58
Grain weight	1.384	**	**	**	**	**	ns	**	*	ns	6.49
NC-Gr	1.486	ns	**	ns	**	ns	ns	**	ns	ns	5.15
KC-Gr	1.805	ns	**	ns	**	ns	ns	**	ns	**	13.51
PC-Gr	1.359	ns	**	ns	**	ns	ns	**	ns	**	14.87
CaC-Gr	2.108	**	**	**	**	ns	**	**	**	**	10.54
MgC-Gr	1.757	ns	**	ns	**	ns	ns	**	ns	*	15.24
SC-Gr	2.491	**	**	ns	**	*	ns	**	ns	*	4.72
FeC-Gr	1.125	**	**	ns	**	ns	**	**	*	**	8.92
CuC-Gr	2.286	*	**	**	**	ns	**	**	**	**	9.24
MnC-Gr	2.550	ns	**	ns	**	*	ns	**	ns	ns	15.55
ZnC-Gr	1.636	**	**	*	**	ns	**	**	ns	**	15.70
NC-Sh	1.146	*	**	**	**	**	ns	**	ns	*	14.19
KC-Sh	1.068	ns	**	*	**	ns	ns	**	ns	ns	10.60
PC-Sh	2.795	ns	**	**	**	**	**	**	*	*	11.14
CaC-Sh	1.485	*	**	ns	**	*	ns	**	ns	**	12.28
MgC-Sh	1.136	*	**	*	**	**	ns	**	ns	ns	12.78
SC-Sh	1.155	*	**	**	**	**	ns	**	*	ns	10.96
FeC-Sh	1.587	ns	**	**	**	ns	**	**	**	**	11.73
CuC-Sh	1.543	**	**	**	**	**	**	**	*	*	14.03
MnC-Sh	1.324	ns	**	**	**	ns	ns	**	**	**	11.94
ZnC-Sh	1.307	**	**	**	**	**	**	**	*	**	12.85

^{ns} – not significant by F-test; * - significant by F-test at p<0,05; ** - significant by F-test at p<0,01; CV – coefficient of variation (%). Degrees of freedom field: block (B) – 3; cultivars (C) – 7; doses (D) – 3; Local (L) – 1; B x L – 3; C x D – 21; L x C – 7; L x D – 3; L x C x D – 21; residue – 186.

Abbreviations: Se content in grain (SeC-Gr) and in shoot (SeC-Sh); Se absorption efficiency (SeAE); Se uptake (SeU); N (NC-Gr), S (SC-Gr), P (PC-Gr), K (KC-Gr), Mg (MgC-Gr), Fe (FeC-Gr), Zn (ZnC-Gr), Mn (MnC-Gr), Cu (CuC-Gr) content in the grain; N (NC-Sh), S (SC-Sh), P (PC-Sh), K (KC-Sh), Mg (MgC-Sh), Ca (CaC-Sh); Fe (FeC-Sh); Zn (ZnC-Sh), Mn (MnC-Sh) and Cu (CuC-Sh) content in the shoot.

ARTIGO 3 – EFFECT OF DIFFERENT SELENIUM SOURCES ON ANTIOXIDANT METABOLISM AND MINERAL CONTENT OF SORGHUM GROWN IN TROPICAL SOIL

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EFFECT OF DIFFERENT SELENIUM SOURCES ON ANTIOXIDANT METABOLISM AND MINERAL CONTENT OF THE SORGHUM GROWN IN TROPICAL SOIL

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Abstract

Agronomic biofortification of plants with selenium (Se) is an important strategy to reduce hidden hunger and increase the nutritional intake of this element in humans and animals. Since sorghum is a staple food for millions of people and is also used in animal feed, it becomes a crop with the potential to be biofortified. Thus, this work aimed to compare organic sources of Se with selenate, which has proven efficiency in several cultures, and to evaluate the grain yield, antioxidant system and mineral content of different sorghum cultivars with foliar Se application. The experiments had a 4 x 8 factorial scheme, with four Se sources (control - without Se supply, SeA - sodium selenate, SeB - potassium hydroxy-selenide, SeC - acetylselenide), and eight cultivars (BM737, BRS310, Enforcer, K200, Nugrain320, Nugrain420, Nugrain430 and SHS410), with four replicates. In the greenhouse, Se was applied at a dose of 0.125 mg plant^{-1}, whereas in the field, Se was applied at a Se dose of 10 g ha^{-1}. All cultivars responded positively to foliar fertilization with Se through sodium selenate in the different growing conditions studied. Selenium sources, potassium hydroxy-selenide and acetylselenide, in these studies provided low Se contents and had lower Se uptake and Se absorption efficiency compared with selenate. Selenium fertilization increased grain yield, altered the antioxidant metabolism and mineral content of the studied cultivars.

Keywords: selenate, selenide, food security, food composition.

1. Introduction

Sorghum (*Sorghum bicolor* L. Moench) is the staple food for nearly 500 million people (Raswan et al., 2021). In 2019, sorghum production in Brazil was 2.7 million tons on 818.3 thousand hectares, and world production was about 57.9 million tons on 40 million hectares (FAOSTAT, 2020). Sorghum has resistance to drought, high productivity, low need for mineral nutrition, and low production costs, allowing cultivation in diverse environments worldwide (Adebowale et al., 2020). According to Lopes et al. (2018), sorghum can be added to the diet by consuming sorghum flour, beneficially increasing antioxidant and modulate status in humans. Research has shown that whole sorghum is a source of carbohydrates, fibre, bioactive compounds (phenolic acids and anthocyanins), starch and minerals (Awika and Rooney, 2004).

The mineral content varies depending on the nutritional status of the plants. Nutrients can have synergistic and/or antagonistic relationships with selenium (Se), improving plant nutrition and easing abiotic stresses such as drought, high temperatures, salinity, and heavy metals (Gupta and Gupta, 2017). Selenoproteins act as antioxidants in plant metabolism through the glutathione peroxidase (GSH) pathway and provide an increase in enzymatic (SOD, CAT and APX) and non-enzymatic (ascorbic acid, flavonoids and tocopherols) compounds that act on the reactive oxygen species (ROS) elimination system and cell detoxification (Lanza and Reis, 2021). Different plant species have variable physiological responses to Se. When growing in seleniferous soils, some plant species are tolerant to Se and accumulate this element in high concentrations. However, most plants are sensitive to Se and accumulate this element in very low concentrations (Terry et al., 2000).

Selenium content in the soil is inconsistent worldwide, with soils being classified as poor in Se to seleniferous soils (Chauhan et al., 2019). In Brazil, a similar scenario occurs due to its wide territorial extension. Carvalho et al. (2019) found Se contractions ranging from 22 to 72 µg kg^{-1} in soils of the Brazilian Cerrado. Moreover, Reis et al. (2017) found Se concentration ranging from 0.002 to 0.65 µg kg^{-1}. Soil with low Se concentrations causes a low concentration of this element in plants. Hence, soils with low Se concentrations cause low Se concentrations in plants, so Se deficiency was mainly observed in these areas and occurs in people worldwide (Schiavon et al., 2016). Selenium is an essential micronutrient for animal and human nutrition (Terry et al., 2000). When at adequate doses, it provides beneficial effects due to its biological functions.

The biological functions of Se were carried out through 25 known selenoprotein genes

that encode various parts (Kryukov et al., 2003); these genes are related to thyroid hormone synthesis, antioxidant defense system and immune function (Gandhi et al., 2013) in animals and humans. Recently, Moghaddam et al. (2020) correlated that people with adequate levels of Se had lower mortality risk if detected by SARS-CoV-2.

Dietary Se intake can be complemented by the biofortification of foods, including fertilization and genetic approaches (White and Broadley, 2009). Selenium translocation from the root to the shoot depends on the Se form given. Selenate was much more easily transported than selenite, or organic Se, such as SeMet (Terry et al., 2000). Biofortification of Se through foliar fertilizers containing selenite or selenate has been practiced in countries such as Finland, the United Kingdom, Malawi and China that obtained an increase in the Se content of cultivated grains or by the foliar application selenite or selenate (Eurola et al., 1991; Rayman, 2008; Fang et al., 2008; Chilimba et al., 2012). However, there are few studies with the fertilization of Se organic, mainly with foliar application. Thus, this work aimed to compare organic sources of Se with selenate, which has proven efficiency in several cultures, and to evaluate the grain yield, antioxidant system and mineral content of different sorghum cultivars with foliar Se application.

2. Material and Methods

2.1. Experimental Design

The experiment was carried out in a greenhouse, in a completely randomized design in a 4 x 8 factorial scheme, with four Se sources (control - without Se supply, SeA- sodium selenate, SeB - potassium hydroxy-selenide, and SeC - acetylselenide), eight cultivars (BM737, BRS310, Enforcer, K200, Nugrain320, Nugrain420, Nugrain430 and SHS410) and four replicates, totaling 128 experimental plots. The Se dose was 0.125 mg plant^{-1} and the cultivars used in this study are commercial cultivars planted in Brazil with high grain quality and no tannin (Table 1).

The experiment under field conditions was installed in a randomized block design, with the same factorial adopted in the greenhouse. In the field was applied at a Se dose of 10 g ha^{-1}. The plot consisted of four rows of 4 m linear, spaced 0.6 m apart, totaling 9.6 m² per plot. The two lateral lines were considered as borders. The useful area considered the two central lines, and 0.5 m was discarded at each end, totaling 3.6m² for grain harvesting.

2.1. Growing conditions

The experiments were conducted from February to August 2019, with one experiment in a greenhouse at the Department of Soil Science at the Federal University of Lavras and two field experiments located at the Experimental Farm of the Agricultural Research Company of the State of Minas Gerais (EPAMIG) in Lambari; and the Center for Scientific and Technological Development in Agriculture – Fazenda Muquém/UFLA in Lavras, both cities located in the State of Minas Gerais, Brazil (Fig. 1). The climate in Lavras and Lambari, according to the Köppen climate classification, is Cwa (Martins et al., 2018), which presents characteristics such as mesothermal (hot temperate or subtropical), with winter drought (with precipitation in the driest month <60mm, with the temperature in the coldest month ranging between ≥-3°C and <18°C) and hot summer (with temperature in the hottest month without 22°C).

In the greenhouse, the dystrophic Red-Yellow Latosol (Santos et al., 2018) was used, a sandy clayey loam texture, collected in the 0-20m layer, corresponding to the Typic Haplustox (loam) in the Soil Taxonomy (Soil Survey Staff, 1999). In Lavras, the soil in the experimental area was the Dystrophic Red-Yellow Latosol (Santos et al., 2018), corresponding to an Oxisol according to the Soil Taxonomy (Soil Survey Staff, 1999). In Lambari, the soil of the experimental area was classified as a Melanic Gleysol (Santos et al., 2018) corresponding to a Histosol according to the Soil Taxonomy (Soil Survey Staff, 1999). The soils in this area have a history of agricultural cultivation. A soil sample was taken from the experimental areas before the implementation of the experiments. The soil samples were air-dried, sieved with a 4 mm mesh, and characterized by their main physical and chemical properties, according to Teixeira et al. (2017) (Table S2 – supplementary material).

2.1.1. Greenhouse-grown conditions

Pots with $5dm^{-3}$ of soil were used. Based on the chemical analysis of the soil, the calculation of liming was done by the method of neutralization of Al^{+3} and the increase in the Ca^{+2} and Mg^{+2} contents (Ribeiro et al., 1999). Liming was performed using 2.725 mg CaO_3 and 0.649 mg MgO_3 per dm^{-3} of soil. After 30 days of incubation of the soil with moisture close to 60% of the total pore volume (VTP), the basic fertilization for planting was carried out, which consisted of 200 mg of P + 90.3 mg of N ($NH_4H_2PO_4$), 108.4 mg of K + 44.47 mg of S (K_2SO_4), 5 mg of Zn + 2.44 mg of S ($ZnSO_4.7H_2O$), 1.5 mg of Cu + 0.75 mg of S ($CuSO_4.5H_2O$), 4.0 mg of Mn + 2.33 mg of S ($MnSO_4.H_2O$), 0.5 mg of B (H_3BO_3) and 0.1 mg of Mo ($[NH_4]_6Mo_7O_{24}.4H_2O$) per dm^{-3} of soil.

Before sowing, seeds were treated with Standak Top® and Cruiser® as recommended. Subsequently, ten sorghum (*Sorghum bicolor* L.) seeds were sown per pot, and ten days after seedling emergence, thinning was done, leaving two seedlings per pot. During the sorghum cultivation period, fertilization was carried out to meet the nutritional demand of the plants. After thinning were applied: 0.25 mg of B (H_3BO_3), 0.75mg of Cu ($CuSO_4.5H_2O$), 2mg of Mn ($MnSO_4.H_2O$), 0.05mg of Mo ($[NH_4]_6Mo_7O_{24}.4H_2O$), 2.5 mg of Zn ($ZnSO4.7H_2O$). Furthermore, after 50 days of cultivation, 4 mg of Mn ($MnSO_4.H_2O$) and 5 mg of Fe (EDDHA iron chelate) were applied.

The macronutrients were divided into 14 applications during the cycle, and the 1st application consisted of 25 mg of Ca + 17.5 mg of N ($Ca(NO_3)_2$), 30mg of K and 8.37mg of N (nitrate commercial potassium), 4.13 mg of N (NH_4NO_3). The 2nd application consisted of 25 mg of Ca + 17.5 mg of N ($Ca(NO_3)_2$), 30mg of K and 8.37mg of N (potassium nitrate - commercial), 4.13 mg of N (NH_4NO_3), and 12 mg of Mg ($MgSO_4$). The 3rd, 4th, 5th, 6th, 7th, 11th, and 12th applications consisted of 50 mg of K + 13.95 mg of N (potassium nitrate - commercial) and 36.05 mg of N (NH_4NO_3). The 8th, 9th, 10th, 13th, and 14th applications consisted of 30 mg of K + 8.37 mg of N (potassium nitrate - commercial) and 21.82 mg of N (NH_4NO_3).

The sources of Se diluted in a 0.5% surfactant solution (Assist®) were used to prepare the Se dose of 0.125 mg $plant^{-1}$, and two applications were made. The first foliar application of Se was carried out when the plants were in the flowering stage and the second stage of grain filling. The control treatment received only deionized water containing the surfactant. The foliar sprays in the greenhouse were carried out with a manual sprayer with the previous compression. Greenhouse temperatures were maintained at 28 ± 5°C during the day by an automatic-controlled system. The pots were irrigated, and the soil water content was adjusted daily close to the field capacity.

2.1.2 Field-grown conditions

Before sowing, seeds were treated with Standak Top® and Cruiser® as recommended. Sowing was done in conjunction with planting fertilization, which consisted of 350 kg ha^{-1} of commercial fertilizer formula NPK 8-28-16 ($N\text{-}P_2O_5\text{-}K_2O$). After 40 days, the top dressing was done with 480 kg ha^{-1} of the formula NPK 23-00-11 ($N\text{-}P_2O_5\text{-}K_2O$) with 2.7% S.

The sources of Se diluted in a 0.5% surfactant solution (Assist®) was used to prepare the Se dose of 10 g ha^{-1}, and two applications were made. Solutions with Se for in the field

spraying were applied at the same vegetative stage. In the field, spraying was carried out with a pressurized pump coupled to a carbon dioxide container. A 2 m long bar was connected to the pump for uniform application. Foliar applications were made in the morning to provide more excellent foliar Se absorption.

2.2. Biochemical analysis

Sampling was carried out on the fifth day after the second Se application. The V2 leaves were collected immediately conditioned in liquid nitrogen and stored at -80°C for biochemical analysis. Hydrogen peroxide (H_2O_2), lipid peroxidation (MDA), proteins, and the activities of superoxide dismutase (SOD, EC: 1.15.1.1), catalase (CAT, EC: 1.11.1.6), and ascorbate peroxidase (APX, EC: 1.11.1.11) were quantified.

2.2.1. Hydrogen peroxide (H_2O_2) and lipid peroxidation (MDA)

The total of 0.4 g of leaves was macerated in liquid nitrogen and PVPP (polyvinylpolypyrrolidone), which was homogenized in 1.5mL of 0.1% (w/v) trichloroacetic acid (TCA) and centrifuged at 12000 g for 15 min at 4°C. From the obtained extract, the hydrogen peroxide (H_2O_2) was quantified as described in Velikova et al. (2000) and based on a standard curve with known concentrations of H_2O_2. Lipid peroxidation was determined as described by Buege and Aust (1978), and the results were expressed in nanomoles of malondialdehyde (MDA) per gram of fresh mass.

2.2.2. Extraction, quantification of antioxidant enzymes and protein

To quantify the enzyme activity, 0.2 g of fresh mass was macerated in liquid nitrogen with PVPP (polyvinylpolypyrrolidone) and homogenized with 1.5 mL of buffer solution ((0.1 mol L^{-1} potassium phosphate (pH7.8), 0.1 mmol of EDTA (pH 7.0) and 0.01 mol L^{-1} ascorbic acid)). Then, 13000g were centrifuged for 10 min at 4°C, and the supernatant was collected and stored at -20°C (Biemelt et al., 1998). The supernatant was collected and used for the enzymatic analysis of superoxide dismutase (SOD) (Giannopolitis and Ries, 1977), catalase (CAT) (Havir and Mchale, 1987), ascorbate peroxidase (APX) (Nakano and Asada, 1981), and protein (Bradford, 1976).

2.3. Grain yield

The sorghum grains produced were harvested and weighed to determine grain yield. In

the greenhouse, grain yield was determined by collecting the grain produced by plants per pot and divided by the number of plants. While in the field, grain yield was evaluated by harvesting sorghum plants from the two central rows (useful plot). According to the Seed Analysis Rule (MAPA, 2009), grain moisture was determined, and grain yield was converted into dry weight by a correction of 13% moisture.

2.4. Sample digestion procedure

The plants were separated into grains and shoots and then placed in a cross-airflow greenhouse at a temperature of ± 60°C until they reached constant weight (after ±72 h). After drying, the plants were ground, and then 0.5 g of each sample was taken for digestion by the 3051A method described by the United States Environmental Protection Agency (USEPA, 2007). Each aliquot was digested with 5 mL of HNO_3 in PTFE Teflon ® tubes (CEM Corporation, Matthews, NC, USA). The extract was left to stand overnight at room temperature, and digestion was carried out the following day. For this, the flasks were hermetically sealed and placed in a microwave (CEM brand, model Mars-5), with a temperature adjusted to 175°C and a controlled pressure of 0.76 MPa for 15 minutes. After digestion, the extracts were cooled to room temperature. Then, the final volume of the extract was supplemented with an additional 5 mL of deionized water, and the extracts were transferred to flasks and stored at 5°C until analysis.

2.4.1. Macronutrients and micronutrients determination

The determinations of the S, P, K, Ca, Mg, Fe, Cu, Zn, and Mn contents were carried out by optical emission spectrometry with inductively coupled plasma (ICP-OES), brand Spectro, model Blue (Germany), with correction background. The operating parameters and the sample introduction system were as indicated by the manufacturer: plasma power of 1400 W, cooling gas flow of 12 L min^{-1}, the auxiliary gas flow of 0.8 L min^{-1}, and gas flow in the nebulizer 0.85 L min^{-1}. The gas used was argon with a purity ≥ of 99.99%. The determinations were carried out in a multi-elemental way and, thus, standard solutions were prepared using aliquots of standard stock solutions of 1000 mg L^{-1} of the elements under study. Dilutions for all solutions were made with the solvent used in the procedure. The calibration curve for the proposed method contained at least five standards of known concentration. The spectral line for each determining element was shown in table S3 - supplementary material. A sample of standard reference material (Peach Leaves SRM1547)

for plant material was included in each batch for quality control purposes, along with a blank sample. The accuracy of the analytical procedure was verified by analyzing the standard reference material and the results found are following the certified values and the recovery ≥ of 72%.

The total N content was determined by sulfuric digestion and Kjeldahl distillation (Malavolta et al., 1997). It also used a sample of Peach Leaves standard reference material (SRM1547) in each digestion batch for quality control purposes, along with a blank sample. The average N recovery in this material was 116.3% (±1.93 s.e.m.).

2.4.2. Selenium (Se)

Selenium in the digested samples were analyzed by GFAAS (Atomic Absorption Spectrometry with Zeeman background correction and EDL lamp for Se; AAnalyst ™ 800 AAS, Perkin Elmer). A standard solution containing 1 g kg^{-1} Se (98% purity, Fluka, Buchs, Switzerland) was used to prepare the calibration curve for Se determination by GFAAS. A sample of standard reference material (White Clover - BCR 402, Institute for Reference Materials and Measurements, Geel, Belgium) for plant material was included in each digestion batch for quality control purposes, along with a used blank sample to calculate the limits of detection and quantification. The mean recovery of Se in this standard reference material (white clover) was 92.9% (n = 10, [Se] = 6.57 mg kg^{-1}).

The limits of detection (LOD) and quantification (LOQ) were established using ten blank extracts following the same procedure adopted with the samples. Values were calculated three and ten times the standard deviation of ten blank extracts to determine LOD and LOQ, respectively (Khan et al., 2013). In the analysis of the materials under study, the LOD was 52 µg kg^{-1} of extract Se and the LOQ was 173 µg kg^{-1} of extract Se. Undetected values (control treatment) were replaced by half the LOD (½LOD) (Gillespie et al., 2010).

2.6. Efficiency nutritional of Se

To study the efficiency of Se use by sorghum plants, mathematical expressions of nutritional efficiency concepts proposed by several researchers were applied. Selenium uptake (SeU) was expressed in mg $plant^{-1}$ for the result of the experiment conducted in the greenhouse because production is quantified in g $plant^{-1}$, and it was expressed in mg kg^{-1} for the result of the field experiments because productivity is expressed in kg ha^{-1} (Ducsay et al., 2016; Lara et al., 2019). The absorption efficiency of Se doses applied (SeAE) was expressed

in % (Lara et al., 2019). The equations are described below.

$$SeU = \text{Se content in grain (mg hg}^{-1}\text{) x grain yield} \quad \text{(Eq. A1)}$$

$$SeAE = \{(SeU_{treated} - SeU_{control}) *100\} / \text{Se supply (g ha}^{-1}\text{)} \quad \text{(Eq. A2)}$$

2.8. Statistical analysis

Initially, the data obtained in the greenhouse were analyzed for normality by the Shapiro-Wilk test ($p \geq 0.05$), homoscedasticity by the Barlet test ($p \geq 0.05$), and the independence of residues by the Durbin test. Watson ($p \geq 0.05$). Then they were submitted to an analysis of variance. Data from field experiments were introduced to an individual analysis by experiment in the same way as a greenhouse. After accepting the assumptions for the joint analysis of experiments, a joint analysis of variance was performed with the two field experiments, considering simultaneously all the experiments developed in Lavras and Lambari (Pimentel-Gomes, 2009). Means were compared using the Scott-Knott test $p \leq 0.05$.

According to Cao et al. (1999), the data were standardized because the variables are measured in very different units before the samples are agglomerated. Principal component analysis (PCA) was performed to report the variation in doses for each selenium source. This analysis allowed the characterization of the variables that most discriminated against the structural characteristics in each treatment. Thus, the initial set of variables started to be described by two new orthogonal latent variables, making it possible to locate them in two-dimensional figures (Hair Junior et al., 2009). Furthermore, the simple linear relationship between the variables was carried out through a simple Pearson's correlation. The statistical analyses and the graphs were carried out with the software R (R Core Team, 2020).

3. Results

In greenhouse conditions, sorghum plants had a response dependent on the interaction between cultivars and Se sources ($p \leq 0.05$) for the variables evaluated in antioxidant metabolism, grain yield, macro and micronutrient content in grain and shoot, except for Mn in the shoot. Grain weight and Mn content in shoot differed significantly ($p \leq 0.05$) among cultivars (Table S4 –supplementary material). In field-grown conditions, the joint analysis of the experiments showed that the differences between the cultivation sites were significant ($p \leq 0.01$) for all variables in the studies. The interaction between Locations, Cultivars and Se sources (L x C x S) influenced ($p \leq 0.05$) the responses of the variables grain yield, P, K, Ca, Mg, Fe, and Zn content in grain, S, P, Ca, Mg, Fe, Zn, Cu, and Mn in the shoot. The double

interaction between Locations and cultivars (L x C) and Locations and Se sources (L x S) significantly influenced ($p \leq 0.05$) the variables grain weight, S and Mn content in grain, to N content in the shoot. The interaction between Cultivars and Se sources (C x S) and Locations and cultivars (L x C) influenced ($p \leq 0.05$) the response of the variables, N and Cu content in grain. The K content in the shoot was answered as a function of the interaction between Location and Cultivars (L x C) (Table S5 – supplementary material)

3.1. Antioxidant metabolism

The lowest H_2O_2 content occurred with K200 with SeA. The K200 with the control treatment, SeA, and SeB had lower H_2O_2 content 42.58, 60.35, and 57.36%, respectively, than with the SeC. BRS310 had reductions of 26.63, 19.19 and 28.81% in the H_2O_2 content of the control treatment, SeA and SeC compared with SeB. Nugrain420 had lower H_2O_2 content 48.81 and 39.22%, with SeB and SeC compared with SeA. Nugrain320 with the SeB had a lower H_2O_2 content 33.38, 43.41 and 41.14% lower than the control treatment, SeA and SeC. Nugrain430 with SeC had a 26.08, 14.82, and 21.48% reduction in H_2O_2 content with the control treatment, SeA, and SeB, respectively (Fig. 2A).

Selenium decreased lipid peroxidation, that is, the MDA content, in SHS410 and Nugrain420. The SeA, SeB, and SeC reduced by 36.08, 26.49 and 21.01%, respectively, the MDA content compared with the control treatment in the SHS410. MDA content had 14.52, 27.42 and 13.38% reductions with SeA, SeB and SeC, respectively, compared with the control treatment in Nugrain420. BRS310 reduced MDA content of 25.20, 29.43 and 36.87% with the control treatment, SeB and SeC, respectively, concerning the SeA. The control treatment, SeA and SeC reduced by 45.22, 47.19 and 37.13%, respectively, the MDA content concerning SeB in K200. Nugrain320 had lower MDA content with the control treatment, SeA and SeB, and were 31.05, 27.02 and 32.34%, respectively lower than with SeC. Nugrain430 reduced 39.40 and 28.98% MDA content with the control treatment and SeB, respectively, concerning SeC (Fig. 2B).

The main effects observed in SOD were the increases with the SeA of 13.99, 24.34 and 20.06% concerning the control treatment, SeB and SeC, respectively in BM737. Moreover, K200 had SOD increments of 18.19, 22.55, and 21.81% in the control treatment, SeA, and SeC compared with the SeB (Fig. 2C).

The highest CAT activity occurred in the Enforcer with the SeB, which increased by 43.99, 26.27, and 2176% the control treatment, SeA and SeC, respectively. Nugrain320 with

the control treatment, SeA and SeB had increases in CAT activity of 43.69, 37.15 and 30.90% concerning SeC. The same occurred with SHS410, which had increases in CAT activity of 39.57, and 33.01 and 29.44% with the control treatment, SeA and SeSC19 concerning SeC. Nugrain420 responded positively to Se fertilization since the SeA, SeB and SeC increased CAT activity by 25.80, 24.75, 22.33%, respectively, compared with the control treatment. The CAT activity in BRS310 was 14.05, 21.42 and 20.78% higher with the control treatment, SeA and SeC, respectively, concerning SeB. Nugrain430 had higher CAT activity than the control treatment and was 33.68 and 41.76% higher than SeB and SeC, respectively (Fig. 2D).

The highest activity of APX occurred with the SeA in SHS410, which was 15.03, 47.49 and 56.94% higher than the control treatment, SeB and SeC, respectively. The SeA, SeB and SeC increased by 19.48, 30.21 and 24.04%, respectively, compared with the control treatment in BRS310. Nugrain320 with the SeA, SeB and SeC had increases in APX activity of 36.46, 29.02, 32.76%, respectively, concerning the control treatment. K200 had increases in APX activity of 55.51, 57.98 and 43.95%, respectively, concerning SeB. Nugrain420 had greater activity on APX in the control treatment, which was 18.12 and 24.60% higher than the SeB and SeC, respectively (Fig. 2E).

The SeB increased the protein content by 12.60, 13.14, and 18.80% compared with the control treatment, SeA and SeC, respectively, in Nugrain420. The protein content in Nugrain430 with SeB increased by 16.49, 19.97 and 36.88% compared with the control treatment, SeA and SeC, respectively. BM737 had increases in protein content of 28.52, 35.60 and 31.08% with the control treatment, SeA and SeB compared with SeC. K200 had a decrease in protein content of 22.67, 30.48 and 19.76% with SeA, SeB and SeC compared with the control treatment (Fig. 2F).

3.2. Selenium and nutritional efficiency

With the application of organic sources of Se, the levels of Se observed in the leaves were low. The levels of Se in the grain when applied to the sources of organic Se were below the limit of detection and limit of quantification, as well as the control treatment. Foliar application of Se with the SeA (selenate) source increased the Se content in the grain and in the greenhouse shoot (Fig. 4A and 4B). In the field, the result in the different cultivation environments (Lambari and Lavras) was similar to the results obtained in the greenhouse. And the highest Se content in grain and shoot (Fig. 5A and 5B) also occurred with the application of SeA. These results implied that SeU and SeAE were also better in all cultivars

than when SeA was applied, both in the greenhouse (Fig. 4C and 4D) and in the field (Fig. 5C and 5D).

3.4. Grain yield

The grain yield in greenhouse-grown plants varied among cultivars and had a positive response from Se fertilization. The SeB in BM737 increased by 15.26, 15.21 and 21.50% compared with the control treatment, SeA, SeC, respectively. In BRS310, the SeB increased by 21.44, 16.55, and 28.90% concerning the control treatment, SeA and SeC, respectively. The Enforcer had increases of 23.42, 23.42 and 17.51% with the control treatment, SeB and SeC, respectively, concerning SeA. Moreover, K200 had increments of 10.17, 15.30 and 17.07% with control treatment, SeB and SeC, respectively, concerning SeA. The increments in Nugrain320 with SeC were 17.69, 24.75 and 21.44% concerning the control treatment, SeA and SeB. Nugrain430 with SeC had a higher grain yield by 20.83, 13.98 and 17.16% than the control treatment, SeA and SeB. The SHS410 with SeC had increments of 22.76, 10.14 and 12.70% compared with the control treatment, SeA and SeC, respectively. Nugrian420 had 16.13 and 11.00% increments with SeC compared with SeA and SeB and did not differ from the control treatment (Fig. 3F).

In field-grown conditions, the highest grain-yield occurred in Lavras. In Lavras, among the eight cultivars of the only BRS310, there was no significant effect of Se sources. BM737, Enforcer, Nugrain420 and SHS410 had higher grain yields with SeB. BM737 with SeB had increments of 14.64 and 20.58%, and SeC had increments of 9.29 and 15.61% concerning the control treatment and SeA, respectively. The Enforcer had 26.53, 30.25 and 27.46% increments with the control treatment, SeB and SeC, respectively, concerning SeA. Nugrain420 with SeA had increments of 5.34 and 11.34%, and SeB had increments of 6.80 and 16.70% concerning the control treatment and SeC, respectively.

The SHS410 had increments of 16.60, 23.55 and 20.86% with the SeA, SeB and SeC, respectively, concerning the control treatment. Nugrain320 with SeB had increments of 19.61 and 11.20%, and SeC had increments of 25.39 and 17.59% concerning the control treatment and SeA, respectively. The K200 with the control treatment had increments of 12.23 and 10.11%, and SeC had 88.34 and 9.52% concerning SeA and SeB, respectively. Nugrain430 had increments of 12.42, 12.39 and 7.60 with the control treatment, SeA and SeC, respectively, concerning SeB. Among the eight cultivars in Lambari, only three had significant responses concerning Se sources. The BM737 with SeC had increments of 23.98,

31.75 and 15.00% concerning the control treatment, SeA and SeB, respectively. The Enforcer with the SeA, SeB and SeC had increments of 32.28, 28.28 and 21.79%, respectively, concerning the control treatment. SeC and control treatment had 22.65 and 19.94% increments compared with SeA and control treatment, respectively (Fig. 4F).

3.6. Macronutrient's content

3.6.1. Nitrogen and Sulfur

In greenhouse-grown conditions, the N content in the grain had similar results with crude protein. The main differences between Se sources and cultivars occurring are BM737, BRS310, Enforcer, K200, SHS410 and Nugrain420. Nugrain320 and Nugrain430 did not have significant responses to different Se sources (Fig. 5A). The N content in the shoot in the Enforcer with SeA had increments of 13.85, 23.16 and 16.05% concerning the control treatment, SeB and SeC, respectively. In BM737, SeA had increases of 16.59 and 15.42% concerning SeB and SeC. The control treatment of SHS410 had a reduction of 9.43, 14.86, 20.29% concerning SeA, SeB and SeC, respectively. The BRS310, K200, Nugrain320, Nugrain420 and Nugrain430 did not have N content in the shoot influenced by the Se (Fig. 5B). In field-grown conditions, N content in grain was higher in all cultivars in Lambari than in Lavras. Moreover, the highest N content in grain occurs with BRS310 and SHS410. Regardless of the cultivation location, the main differences observed between Cultivars and Se Sources occurred with BM737 and Nugrain430 (Fig. 6A).

The S content in the grain under greenhouse-grown conditions had increments with the SeC in BM737, which was higher 16.96, 16.60 and 8.04% concerning the control treatment, SeA and SeB, respectively. SHS4100 with SeC had increments of 7.30 and 9.56% compared with SeA and SeB. The Enforcer with SeA had increments of 13.67, 17.41 and 7.46% compared with the control treatment, SeB and SeC, respectively. Nugrain420 had increments of 8.51, 6.96, 8.69% with SeA, SeB, and SeC compared with the control treatment. BRS310, K200, Nugrain320 and Nugrain430 do not influence Se sources on the S content in the grain (Fig. 5C). The S content in the shoot in the greenhouse had increments in BM737 with the SeC in 12.05, 15.71, 17.72% concerning control treatment, SeA, and SeB. The Enforcer with the SeA had increases of 33.13, 27.66 and 18.89% concerning the control, SeB and SeC, respectively. SHS410 had increments of 19.07, 19.20 and 20.78% with SeA, SeB and SeC, respectively, in relation to the control treatment. The S content in the shoot of the BRS310, K200, Nugrain320, Nugrain420 and Nugrain430 was not influenced by the Se sources (Fig.

5D).

For the S content in the shoot in field-grown sorghum in Lambari, the main differences occurred with BRS310 and SHS410. BRS310 with the control treatment increased with 13.18 and 10.43% and with SeC increased with 16.53 and 13.88% concerning SeA and SeB, respectively. Furthermore, SHS410 with SeB had increments of 22.69 and 20.15%, and SeC had increments of 23.38 and 20.86% concerning the control treatment and SeA, respectively. In Lavras, the main differences occurred with Enforcer and SHS410. The Enforcer with the control treatment had increments of 15.32 and 13.60%, and SeB had 18.02 and 16.35% concerning SeA and SeC, respectively. SHS410 had increments of 21.80, 20.10 and 17.41% with the control treatment, SeA and SeB respectively in relation to SeC (Fig. 6D).

3.6.2. Phosphorus and Potassium

In the greenhouse, P content in grain had increased with the SeA in BM737 of 13.45, 22.99 and 24.41% concerning the control treatment, SeB and SeC, respectively. BRS310 had increases of 21.28 and 16.99% with SeA and SeC compared with the control treatment. The Enforcer had increments of 21.37 and 27.41% with SeA and SeC concerning the control treatment. Nugrain430 with the control treatment, SeA and SeB had increments of 26.86, 28.45 and 23.29%, respectively, concerning SeC. The K200 had a reduction of 16.44 and 21.91% with SeB and SeC concerning control treatment (Fig. 5E). The P content in the shoot of greenhouse-grown plants treated with SeB and SeC decreases by 49.32 and 38.16% with BM737 and 25.97 and 36.12% with Nugrain420 compared with the control treatment. The SeA increased by 22.48, 32.06 and 39.34% compared with the control treatment, SeB and SeC, respectively. SHS410 had increments of 43.93, 33.85 and 37.59% with the control treatment, SeA and SeB compared with SeC (Fig. 5F).

In field-grown conditions, the P content in grain increased with Se fertilization. In Lambari, the Enforcer with SeB had increments of 24.51 and 32.88%, and SeC had increments of 32.40 and 39.89% concerning the control treatment and SeA, respectively. Nugrain320 with SeB had increments of 17.70, 15.14 and 18.85% compared with the control treatment, SeA and SeC, respectively. The SHS410 with SeB had increments of 20.21 and 11.07% and with SeA had increments of 17.23 and 7.74% concerning the control treatment and SeC, respectively. In Lavras, BRS310 with SeB had increments of 20.08 and 20.27%, and SeC had increments of 18.37 and 18.57% concerning the control treatment and to SeA,

respectively (Fig. 6E).

The P content in the field-grown shoot in Lambari had increments of 14.02, 15.97 and 17.95% with the control treatment, SeB and SeC, respectively, concerning the SeA in the Enforcer. Nugrain430 with SeA had 18.76 and 11.09% increments and SeB increments of 20.84 and 13.37% concerning the control treatment and SeC, respectively. The SHS410 with SeA had increments of 14.59 and 11.77%, and SeC had increments of 20.12 and 17.39% concerning the control treatment and SeB, respectively. In Lavras, the main difference occurred with SHS410, which had increments of 18.16 and 28.94% with the control treatment, and with SeB had increments of 15.93 and 26.99% concerning SeA and SeC, respectively (Fig. 6F).

In greenhouse-grown plants, K content in grain had increases of 11.67 and 11.78% with SeA and SeC concerning the control treatment in BRS310. Nugrain430 had increments of 26.24, 25.43 and 22.42% with the control treatment, SeA and SeB, respectively, concerning SeC (Fig. 5G). The K content in the shoot of in greenhouse-grown sorghum had increases in the control treatment of 16.93 and 10.66% concerning SeB and SeC in BM737 and 11.37 and 13.03% concerning SeA and SeB in BRS310. The SeA in the Enforcer increased by 16.37, 23,016 and 13.65% compared with the control treatment, SeB and SeC, respectively. SHS410 had increments of 15.56, 1.25 and 13.59% with the control treatment, SeA and SeC respectively in relation to SeC (Fig. 5H).

In field-grown sorghum, the main effects of Se in K content in grain in Lambari occurred with Enforcer and Nugrain320. The Enforcer with SeB had increments of 22.21 and 30.33%, and SeC had 29.51 and 36.87% concerning the control treatment and SeA, respectively. Nugrain320 with SeB had increments of 14.77, 13.77 and 17.34% with the control treatment, SeA and SeC, respectively. In Lavras, BRS310 with SeB had increments of 18.58 and 21.09%, and SeC had increments of 17.00 and 19.56% concerning the control treatment and to SeA, respectively. Furthermore, the Enforcer with SeA, SeB and SeC had increments of 25.14, 20.56 and 29.27%, respectively, concerning the control treatment (Fig. 6G).

3.6.3. Calcium and Magnesium

In greenhouse-grown conditions, the Ca content in grain with the SeC had increases of 35.58, 35.58 and 23.08% concerning the control treatment, SeA and SeB, respectively in BM737. SHS410 with SeC had increments of 26.53 and 32.65% compared with SeA and

SeB. The SeB in K200 increased by 28.78 and 21.21% compared with SeA and SeC. Nugrain430 had increments in the control treatment of 43.33, 45.00 and 48.33% compared with SeA, SeB and SeC, respectively (Fig. 5I). The Ca content in the shoot with the SeB had increments of 15.29, 8.85 and 15.74% concerning the control treatment, SeA and SeC in BRS310. Nugrain430 with SeC had increments of 19.57, 13.97 and 12.38% compared with the control treatment, SeA and SeB, respectively. The SeB and SeC increased by 10.51 and 18.02% compared with the control treatment in K200. The Enforcer had increments of 12.94, 21.84 and 21.26% with the control treatment, SeA, SeC, respectively, concerning SeB. The Nugrain420 increased the control treatment by 12.82 and 11.72% concerning the SeB and SeC (Fig. 5J).

In field-grown conditions, the Ca content in grain had variation concerning cultivars and Se sources. In Lambari, the K200 with SeA had increments of 10.26, 21.79 and 14.10% concerning the control treatment, SeB and SeC, respectively. BRS310 had increases in the control treatment of 41.23, 30.65 and 37.63% in relation to SeA, SeB and SeC, respectively. The Enforcer with the control treatment, SeB and SeC, had increments of 20.51, 27.90 and 32.61%, respectively, compared with SeA. Nugrain320 with SeB had increments of 15.48, 24.73 and 21.50% compared with the control treatment, SeA and SeC, respectively. Nugrain420 with the control treatment, SeA and SeC had increments of 28.19, 21.27 and 25.34% concerning SeB, respectively. Nugrain430 with SeB had increases of 11.51, 17.27 and 22.70% compared with the control treatment, SeA, and SeC, respectively.

In Lavras, Ca content in grain varied significantly with BRS310, BM737 and SHS410. BRS310 with SeC had increases of 38.89, 38.83 and 21.28% compared with the control treatment, SeA and SeB, respectively. BM737 with SeA had increments of 46.45 and 43.72%, and SeB had increments of 57.94 and 55.79% concerning the control treatment and SeC, respectively (Fig. 7A). Calcium content in the shoot in field-grown sorghum had no significant differences in Lambari. In Lavras, the BM737 with SeC had increments of 12.02, 18.80 and 9.39% concerning the control treatment, SeA and SeB, respectively. The SHS410 with the control treatment, SeA and SeB had increments of 18.91, 25.18 and 21.69% respectively, in relation to SeC (Fig.7B).

In the greenhouse, the Mg content in grain with the SeA had increases of 31.25 and 22.72% in BM737 and 19.69 and 24.65% in K200 concerning SeB and SeC. BRS310 with SeA had increases of 31.77, 29.39 and 15.76% compared with the control treatment, SeB and SeC, respectively. The Enforcer had increases of 23.07 and 18.09% with SeA and 31.81 and

27.41 with SeC compared with the control treatment and SeB. Nugrain430 with the control treatment, SeA and SeB had increases of 26.97, 29.56 and 24.19%, respectively, over SeC (Fig. 5K). In the shoot with SeA, Mg content had 11.33 and 21.19% increments compared with SeB and SeC in Nugrain420. The Enforcer with the SeA had increments of 19.25 and 28.59%, and SeC had increments of 11.24 and 21.54% concerning the control treatment and SeB (Fig. 5L).

In field-grown conditions, Mg content in grain was little affected. In Lambari, Enforcer with SeB had increments of 24.08 and 33.71%, and SeC had increments of 34.18 and 42.53% concerning the control treatment and SeA, respectively. Nugrain320 with SeB had increments of 15.86, 15.86 and 19.02% compared with the control treatment, SeA and SeC, respectively. In Lavras, BRS310 with SeB had 19.18 and 21.04%, and SeC had increments of 18.09 and 19.97% concerning the control treatment and SeA, respectively (Fig. 7C). Magnesium content in the shoot in field-grown conditions had significant differences with K200 in Lambari and SHS410 in Lavras. In Lambari, K200 with the control treatment had increments of 17.40 and 18.64%, and SeB had increments of 15.95 and 17.21% concerning SeA and SeC, respectively. In Lavras, the SHS410 with control treatment, SeA and SeB had increases of 15.07, 12.63 and 18.70%, respectively, concerning SeC (Fig. 7D).

3.7. Micronutrient's content

3.7.1. Iron and Zinc

The Fe content in grain in the greenhouse-grown was higher with the SeA in BRS310, which was 27.64, 21.89 and 19.2% higher than the control treatment, SeB and SeC, respectively. The SeA increased by 21.11 and 12.63% in BM737, and 16.39 and 2720% in Nugrain430 concerning SeB and SeC, respectively (Fig. 5M). The Fe content in shoot in the greenhouse-grown was higher with SeB in SHS410, which was 37.61, 34.08 and 32.39% than the control treatment, SeA and SeC, respectively. Nugrain320 with SeA had increments of 8.74, 9.60 and 11.67% compared with the control treatment, SeB and SeC, respectively (Fig. 5N).

In the field, Fe content in grain had significant differences with Se fertilization in BM737, Enforcer, Nugrain320 and Nugrain420 in Lambari. In Lambari, BM737 with SeA had increments of 11.04 and 19.13%, and with SeB had increments of 11.08 and 19.16% concerning the control treatment and SeC, respectively. The Enforcer with SeB had increments of 19.58 and 25.30%, and with SeC had increments of 22.27 and 27.80%

concerning the control treatment and SeA, respectively. Nugrain320 with SeB had 17.00, 16.00 and 12.97% increments compared with the control treatment, SeA and SeC, respectively. Nugrain420 with SeC had increases of 11.44, 8.60 and 17.25% compared with the control treatment, SeA and SeB, respectively.

In Lavras, the K200 with SeB had increments of 12.92, 12.76 and 22.83% concerning the control treatment, SeA and SeC, respectively. BRS310 with SeB had increments of 30.20, 23.11 and 19.92% with the control treatment, SeA and SeC, respectively. The SHS410 with SeB had increments of 12.51, 26.11 and 19.88% concerning the control treatment, SeA and SeC, respectively. BM737 with SeA had increments of 20.92 and 17.88%, and SeB had 18.56 and 15.43% concerning the control treatment and SeC, respectively. The Enforcer increased by 21.92, 27.48 and 29.14% with the SeA, SeB and SeC, respectively, concerning the control treatment. Nugrain320 with the control treatment had increments of 13.39, 12.16 and 12.75% compared with the control treatment (Fig. 8A).

The Fe content in the shoot of field-grown sorghum had significant differences between Se sources and cultivars in Lambari and Lavras. In Lambari, the K200 with the control treatment, SeA and SeB had increments of 27.12, 20.08 and 26.80%, respectively, concerning SeC. BRS310 with SeA had increments of 10.95, 12.57 and 15.06% in relation to the control treatment, SeB and SeC, respectively. SHS410 with SeB had increments of 11.40 and 12.33%, and SeC had 17.98 and 18.86% concerning the control treatment and SeA, respectively. The BM737 with SeA had increments of 32.46, 24.22 and 15.32% concerning the control treatment, SeB and SeC, respectively. The Enforcer with the control treatment, SeB and SeC, had increments of 28.66, 21.88, 30.35%, respectively, concerning SeA. Nugrian320 with SeA had increments of 9.28 and 24.34%, and SeC had increments of 22.03 and 34.97% concerning the control treatment and SeB, respectively. Nugrain430 with SeB had increments of 14.62 and 10.85%, and SeC had increments of 23.92 and 20.57% concerning the control treatment and SeA, respectively.

In Lavras, the SHS410 with the control treatment had increments of 13.11 and 30.70%, and the SeA had increments of 12.74 and 30.40% concerning the SeB and SeC, respectively. The BM737 with SeB had increments of 7.57 and 15.74%, and with SeC had increments of 14.84 and 22.37% concerning the control treatment and SeA, respectively. The Enforcer with SeB had increases of 14.58, 21.98 and 18.96% compared with the control treatment, SeA and SeC. Nugrain320 with the control treatment had increments of 21.81, 33.60 and 28.21% in relation to SeA, SeB and SeC, respectively. Nugrain420 with SeC had

increases of 16.43, 30.10 and 33.10% compared with the control treatment, SeA and SeB, respectively. Nugrain430 with the control treatment, SeB and SeC had increments of 27.86, 19.60 and 29.17%, respectively, concerning SeA (Fig. 8B).

Zn content in the grain in the greenhouse-grown was higher with BRS310 with SeA, which was 23.73 and 17.92% higher than the control treatment and SeB. The SeA increased by 22.30 and 18.31% concerning the control treatment and SeB in Enforcer and 20.55 and 14.57% of increments concerning SeB and SeC in K200. BM737 had increments with SeA of 15.84, 13.92 and 20.13% compared with the control treatment, SeB and SeC, respectively (Fig. 5O). Zinc content in the shoot of greenhouse-grown plants was higher in SHS410 with SeA, which was 11.65 and 13.%17 higher than SeB and SeC. The SeC in Nugrain320 increased by 15.42 and 13.40% compared with SeA and SeB. The BRS310 with the SeC had increments of 22.75, 14.06 and 20.77% concerning the control treatment, SeA and SeB, respectively. The Enforcer with SeC had increments of 21.02 and 14.52% and with SeA increments of 18.89 and 12.22% concerning the control treatment and SeB. K200 had 18.10, 16.02 and 20.19% increments with the control treatment, SeA and SeC, respectively, concerning SeB (Fig. 5P).

Zn content in grain was little affected under field-grown conditions. In Lambari, BRS310 with control treatment had increments of 20.49, 13.44 and 21.44% in relation to SeA, SeB and SeC, respectively. The Enforcer with SeB had increments of 27.04 and 38.32%, and with SeC had increments of 28.11 and 39.23% concerning the control treatment and SeA, respectively. In Lavras, BRS310 with SeB had increments of 17.36 and 21.01%, and SeC had increments of 18.42 and 22.03% concerning the control treatment and SeA, respectively (Fig. 8C).

The Zn content in the field-grown shoot in Lambari Se sources provided significantly different responses only on the Enforcer and SHS410. The Enforcer with the control treatment, SeB and SeC, had increments of 17.79, 22.51 and 18.92%, respectively, concerning SeA. Moreover, the SHS410 with SeC had increments of 19.71, 17.17 and 28.08% concerning the control treatment, SeA and SeB, respectively.

In Lavras, the K200 with SeC had increments of 26.85, 38.77 and 25.43% with the control treatment, SeA and SeB, respectively. The SHS410 with the control treatment had increments of 17.25 and 18.13%, and SeB had increments of 9.77 and 10.73% concerning SeA and SeC, respectively. The Enforcer with SeA had increments of 22.60 and 25.49%, and with SeB had increments of 14.41 and 17.61% concerning the control treatment and SeC,

respectively. Nugrain420 with SeC had increments of 18.92, 20.27 and 23.03% compared with the control treatment, SeA and SeB, respectively. Nugrain430 with SeB had increments of 40.19, 30.41 and 35.29% with the control treatment, SeA and SeC, respectively (Fig. 8D).

3.7.2. Copper and Manganese

The Cu content in the grain in the greenhouse-grown with the SeA in BM737 had increments of 24.93, 44.40 and 40.93% concerning the control treatment, SeB and SeC, respectively. BRS310 had increments of 37.24, 49.97 and 29.26% with the control treatment, SeB and SeC, respectively, in relation to SeA. Nugrian320 with the SeA had increments of 46.59, 21.35 and 36.59% concerning the control treatment, SeB and SeC. SHS410 had increases of 14.77 and 37.75% with SeA and 13.87 and 37.08% with SeB compared with the control treatment and SeC. Nugrain420 had increments of 38.23, 45.52 and 50.35% with the control treatment, SeB and SeC, respectively, concerning SeA. Nugrain430 had 37.42, 48.76 and 40.91% increments with the control treatment, SeA and SeB, respectively, concerning SeC. The K200 with the SeC had increments of 43.38, 73.39 and 87.96% concerning the control treatment, SeA and SeB (Fig. 5Q).

The Cu content in the shoot in the greenhouse-grown with the SeA had increments of 28.59 and 36.35% in Nugrain430, 32.75 and 51.86% in SHS410, and 60.18 and 49.90% in the BM737 concerning SeB and SeC. The Enforcer with the SeA had increments of 38.52, 40.73 and 15.85% concerning the control treatment, SeB and SeC, respectively. Nugrain320 with the SeB had increments of 20.15 and 19.85% compared with SeA and SeC. Nugrain420 had increased with SeB of 11.16 and 19.46% and with SeC 16.64 and 24.43% compared with the control treatment and SeA, respectively. The K200 with the control treatment, SeA and SeC had increments of 38.38, 44.86 and 39.36%, respectively, concerning SeB. BRS310 had increases in the control treatment of 13.09, 28.63 and 12.95% in relation to SeA, SeB and SeC (Fig. 5R).

The highest Cu content in the grain in field-grown sorghum occurred in Lambari concerning Lavras. In Lambari, the BRS310, Enforcer, K200, Nugrian320, Nugrain420, Nugrain430 and SHS410 had increments of 56.98, 58.94, 67.41, 33.85, 63.38, 77.06 and 44.36% respectively, in relation to Lavras. Regardless of field-grown, BRS310 with SeA had increases of 14.48, 24.70 and 14.48% compared with the control treatment, SeB and SeC, respectively. The Enforcer with SeC had 44.71, 55.02 and 32.80% increments compared with the control treatment, SeA and SeB, respectively (Fig. 8E).

The Cu content in the shoot had significant differences from field-grown ones. In Lambari, K200 with the control treatment had increments of 22.14 and 10.68%, and with SeB had increments of 29.95 and 19.65% concerning SeA and SeC, respectively. BRS310 with the control treatment had increments of 25.26 and 20.77%, and SeC had increments of 33.60 and 29.61% concerning SeA and SeB, respectively. The SHS410 with the control treatment with 38.63 and 22.13% and SeC had increases of 33.33 and 15.41% concerning SeA and SeB, respectively. In Lavras, the Enforcer with SeA, SeB and SeC had increments of 24.24, 25.70 and 18.85%, respectively, concerning the control (Fig. 8F).

In greenhouse conditions, the Mn content in grain in BRS310 had increments of 20.25 and 19.15% with SeA and 13.11 and 11.91% with SeC concerning the control treatment and SeB, respectively. The Enforcer had 20.32 and 19.92% increments with SeA and 14.40 and 13.98% with SeC compared with the control treatment and SeB, respectively (Fig. 5S).

In field-grown conditions, Mn content in shoot varied significantly among field-grown ones. In Lambari, K200 with the control treatment had increments of 16.92 and 18.92%, and SeC had increments of 8.44 and 10.64% concerning SeA and SeB, respectively. BRS310 with the control treatment had increments of 19.51 and 33.05%, and SeC had increments of 21.39 and 34.62% concerning SeA and SeB, respectively. SHS410 with SeC had increases of 13.10, 33.85 and 25.57% compared with the control treatment, SeA and SeB, respectively. BM737 with control treatment had increments of 8.44, 18.15 and 12.39% SeA, SeB and SeC respectively. The Enforcer with the control treatment, SeB and SeC, had increments of 27.47, 30.07 and 31.63%, respectively, concerning SeA. Nugrain320 with SeC had increments of 29.62, 19.72 and 29.35% compared with the control treatment, SeA, SeB respectively. Nugrain420 with SeC had increments of 21.22, 38.58 and 22.85% compared with the control treatment, SeA and SeB. Nugrain430 with control treatment had increments of 12.54 and 30.33%, and SeC had increments of 22.14 and 37.98% concerning SeA and SeB.

In Lavras, BRS310 with SeA had increases of 21.85, 14.60 and 25.76% concerning the control treatment, SeB and SeC, respectively. The BM737 with SeC had increments of 25.05, 24.07 and 17.64% concerning the control treatment, SeA and SeB, respectively. The Enforcer with SeA had increments of 30.50 and 22.10%, and SeB had increments of 19.67 and 9.97% concerning the control treatment and SeC, respectively. Nugrain320 with the control treatment had increments of 20.53, 13.62 and 10.63% in relation to SeA, SeB and SeC, respectively. Nugrain420 with SeC had increments of 16.12, 31.52 and 24.85% with the control treatment, SeA and SeB, respectively (Fig. 8H).

3.8 Principal Component Analysis and Pearson's Correlation

The principal component analysis allowed a better understanding of the general effect of different sources of se and different cultivars on the variables evaluated in this study. The first component (horizontal axis) represented most of the total variation in the three growing environments. The clusters were formed as a function of Se source and cultivars. The first two main components accounted for 51.55% of the total variation in the greenhouse (Fig. 9). It was observed that in the greenhouse, grain yield was influenced by SeB and SeC sources, with emphasis on Nugrain430 and SHS410. However, grouping with the application of Se in the form of SeA indicated a better response from this source in relation to Se content in relation to cultivars. In Lambari (Fig. 10) and Lavras (Fig. 11), the response was 54.05 and 52.18%, respectively, of the total variation. In the cultivated field, it was observed that Se sources also influence productivity. In Lambari, the response was similar to the greenhouse study. However, in Lavras, the overlapping groups indicate little influence of Se sources on productivity.

Pearson's correlation for the response of plants in the greenhouse (Fig. 12) indicated that with an increase in grain yield there was a decrease in CaC-Gr, SOD, CAT, MgC-Sh, SC-Sh, APX, KC-Sh, FeC-Gr, NC-Sh, SC-Gr, CuC-Sh, CuC-Gr, ZnC-Sh, ZnC-Gr, PC-Sh, NC-Gr, MnC-Gr, MgC-Gr, and PC-Gr. Selenium content did not show significant correlation with the other variables. In the field cultivation, the Pearson correlation had a different response between Lambari and Lavras. In Lambari (Fig. 13), the increase in grain yield did not significantly correlate with Se and other nutrients under study. SeC-Gr was negatively correlated with MnC-Sh, MgC-Sh, CuC-Sh, ZnC-Sh, SC-Sh, CuC-Gr. In Lavras (Fig. 14), the increase in grain yield was negatively correlated, indicating a decrease in NC-Gr, FeC-Gr, SC-Sh, PC-Sh, CaC-Sh, FeC-Sh and MgC-Sh. The effects of Se (SeAE, SeU, SeC-Sh and SeC-Gr) occurred mainly in relation to ZnC-Gr, MgC-Gr, PC-Gr, CuC-Gr, and ZnC-Sh which were negatively correlated.

4. Discuss

Plants can stimulate different ways to overcome oxidative stress via the production of reactive oxygen species (ROS) produced as a function of different environmental conditions (Schiavon et al., 2017). The cell defense response involves enzymatic and non-enzymatic antioxidant mechanisms to maintain the low concentration of ROS (Schiavon et al., 2017). In this study, the application of Se promoted contradictory responses among sorghum cultivars,

being observed both increases and decreases in the contents of H_2O_2 and MDA. The observed increases in H_2O_2 and MDA contents may be because a phytotoxic concentration has been reached even if there have been no visible symptoms of phytotoxicity. According to Ríos et al. (2008) when Se is in concentration at the phytotoxicity level, it causes increases in the H_2O_2 content that influence the MDA content.

Selenium also increases the antioxidant capacity of plant cells by increasing the activity of antioxidant enzymes, which contribute to plant resistance to stress situations (Feng at al., 2013). According to Shieber and Chandel (2014), superoxide dismutase (SOD) is the first antioxidant enzyme to act as a barrier against oxidative stress due to the O_2- dismutation reaction to form O_2 and H_2O_2. However, CAT stimulates the degradation of H_2O_2 to H_2O and O_2 due to its high compatibility with this substrate. And APX reduces H_2O_2 to H_2O and O_2 using ascorbate as an electron donor for this reaction (Reis et al., 2017; Silva et al., 2018).

Low doses of Se have shown a protective function against different abiotic stresses, including drought, cold, heat, salinity and UV-B radiation, which also cause oxidative stress (Feng et al., 2013). The beneficial effect of Se on sorghum plants when under high temperature stress was observed by Djanaguiraman et al. (2010) with the foliar application of Se attenuates oxidative stress by intensifying the antioxidant defense mechanism in the sorghum grain, which reduces membrane damage. Soils with low Se contents result in foods with low Se concentrations not being able to supply Se in adequate amounts by animals and humans (Lopes et al., 2017). Therefore, biofortification with Se is necessary, and its effectiveness has been reported by several authors (Broadley et al., 2010; Schiavon et al., 2016; Lidon et al., 2019; Babalar et al., 2019). In this study, foliar fertilization with Se in the form of selenate proved to be more efficient in the biofortification process of sorghum than the other sources tested.

Other Se sources were tested, such as $SeSO_4$, which provided the tallest plant and the largest stem diameter due to Se supply (Qureshi et al., 2021), that is, positive results in sorghum plants. Selenium mobility was assessed by Kikkert and Berkelaar (2013) in Canola and Trigo through the study of the translocation factor and was in the following order: selenate> SeMet> selenite / SeCys. Selenium absorbed by leaves can be transported via active transport via the symplastic route (Carini and Bengtsson 2001). After this process, long-distance transport to other tissues occurs through the phloem vascular system (Shahid et al., 2017). However, the processes of absorption, translocation and distribution of Se are related to several factors, such as: plant species, stages of plant development, form and concentration

of Se, presence of other substances, activity of membrane transporters, and mechanisms of plant translocation (Zhao et al., 2005; Li et al., 2008; Renkema et al., 2012).

Selenate source promoted an increase in Se content in the grain for all cultivars under study, yet attention should be paid to the maximum Se limit that can be ingested. For humans, the daily Se intake for adults ranges from 30 to 70 μg day^{-1} (United States Department of Agriculture, 2005). For animals such as dairy cattle the Se recommendation is 0.3 mg kg^{-1} with dry mass (DM) based diet (NRC, 2001) For beef cattle, broilers, and swine Se recommendation are 0.1 to 0.3, 0.15 and 0.30 mg kg^{-1} of diet (NCR, 1996, 1994, 1998).

The application of Se in low doses can provide an increase in productivity, which may be related to the beneficial effects that Se promotes (Subramanyam et al., 2019). Selenium alters the activity of enzymes such as catalase (CAT) and superoxide dismutase (SOD), providing an anti-senescence effect and an improvement in the antioxidant defense system, which may explain the benefits obtained in productivity (Hasanuzzaman et al., 2020). Ducsay et al. (2016) also observed an increase in wheat production compared with control when 10 g ha^{-1} of selenate was applied via leaf.

Selenium and S have chemical similarities, so S transporters and enzymes are the main means of Se absorption and assimilation, which also affects the N assimilation pathway (Schiavon et al., 2016). The action of Se in the metabolic pathways of S, N and phenol can occur as a function of the dose of Se, selenate/sulfate and the time of exposure to Se fertilization (Schiavon et al., 2016).

The application of Se can interfere with the content of nutrients such as P, K, Mg and Fe due to these elements being abundant in sorghum (Pontieri et al., 2014). The active action of Se was evidenced in Fe absorption (Feng et al., 2009; Feng and Wei, 2012), and can be considered as one of the Se-mediated mechanisms to reduce the metal's toxicity in plants (He et al., 2004). Zinc is a component of the CuZnSOD enzyme, being an important enzyme against ROS attack. It also has an antagonist action with copper and iron, and prevents the oxidation of the sulfhydryl groups of proteins (Yildiz et al., 2019).

5. Conclusion

All cultivars responded positively to foliar fertilization with Se through sodium selenate in the different growing conditions studied. The other sources in studies provided low Se contents and had lower Se uptake and Se absorption efficiency compared with selenate. Selenium fertilization increased grain yield, altered the antioxidant metabolism and mineral

content of the studied cultivars. Therefore, sorghum plants respond positively to foliar application of Se.

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Table 1 – Summary of characteristics of eight sorghum cultivars.

Cultivars	RNC*	PH (cm)	SF (days)	SH (days)	Color grain
BM737	29344	135-145	51-55	120-130	Red
BRS310	18751	115	65	120	Red
Enforcer	30336	120-130	55-60	110-120	Brown
K200	38142	140-160	UN	UN	Red
Nugrain320	38145	130	58-60	110-115	Orange
Nugrain420	40315	150	65-70	125-130	Orange
Nugrain430	36743	140	65-70	125-130	Orange
SHS410	18772	125-135	48-52	120-130	Red

UN – uninformed; PH – plant height; SF – sowing to flowering; SC – sowing to harvest. *RNC-National Registry of cultivars. Details about patent and Maintainer of cultivar can be found at Brazil (2020).

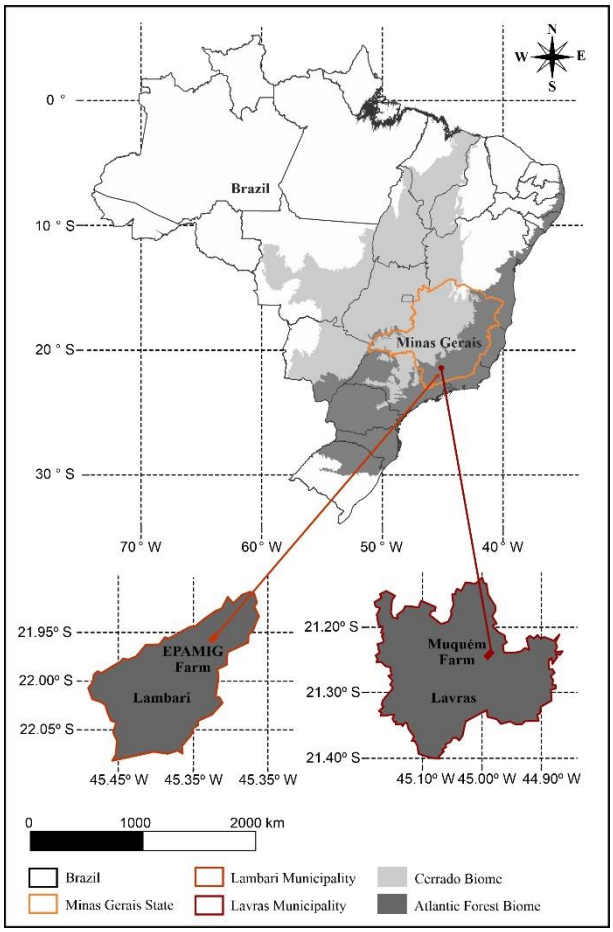

Fig. 1- Location of the experimental area.

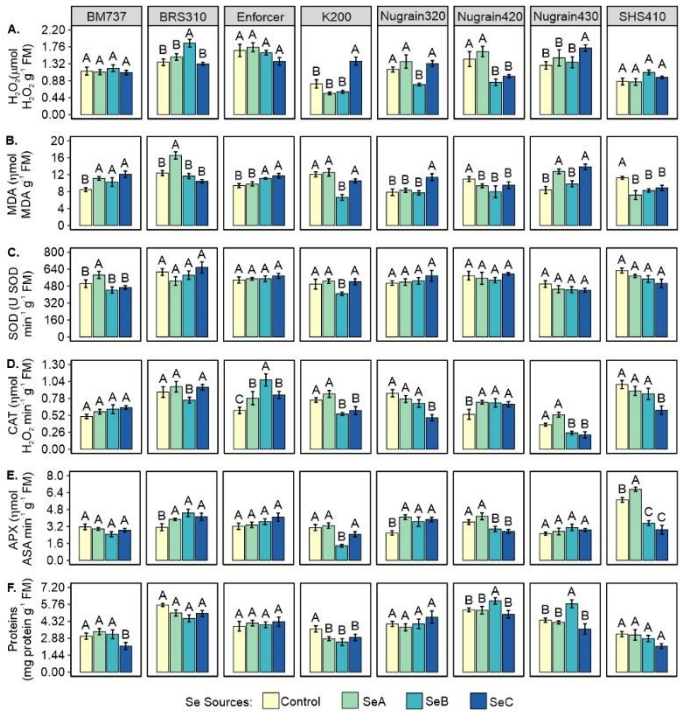

Fig. 2 – Greenhouse-grown conditions. Hydrogen peroxide – H_2O_2 (A), lipid peroxidation by the malondialdehyde content - MDA (B), catalase – CAT (C), ascorbate peroxidase – APX (D), superoxide dismutase – SOD (E), and proteins in extract enzymatic (F). The capital letters compare Se sources in the same cultivar. Different letters indicate significant differences between treatments at a probability level of 5% ($p<0.05$) by test Scott-Knott. The bars show means, and the vertical error bars refer to the standard errors (n=4).

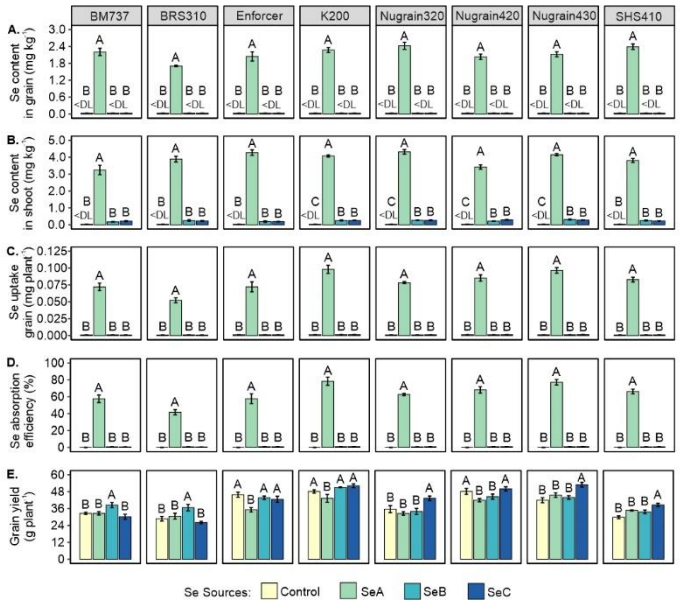

Fig. 3 – Greenhouse-grown conditions. Selenium content in grain (A), Se content in the shoot (B), Se accumulation in grain (C), Se uptake by grain (D), Se absorption efficiency (E), grain yield (F). The capital letters compare Se sources in the same cultivar. Different letters indicate significant differences between treatments at a probability level of 5% ($p<0.05$) by test Scott-Knott. The bars show means, and the vertical error bars refer to the standard errors (n=4).

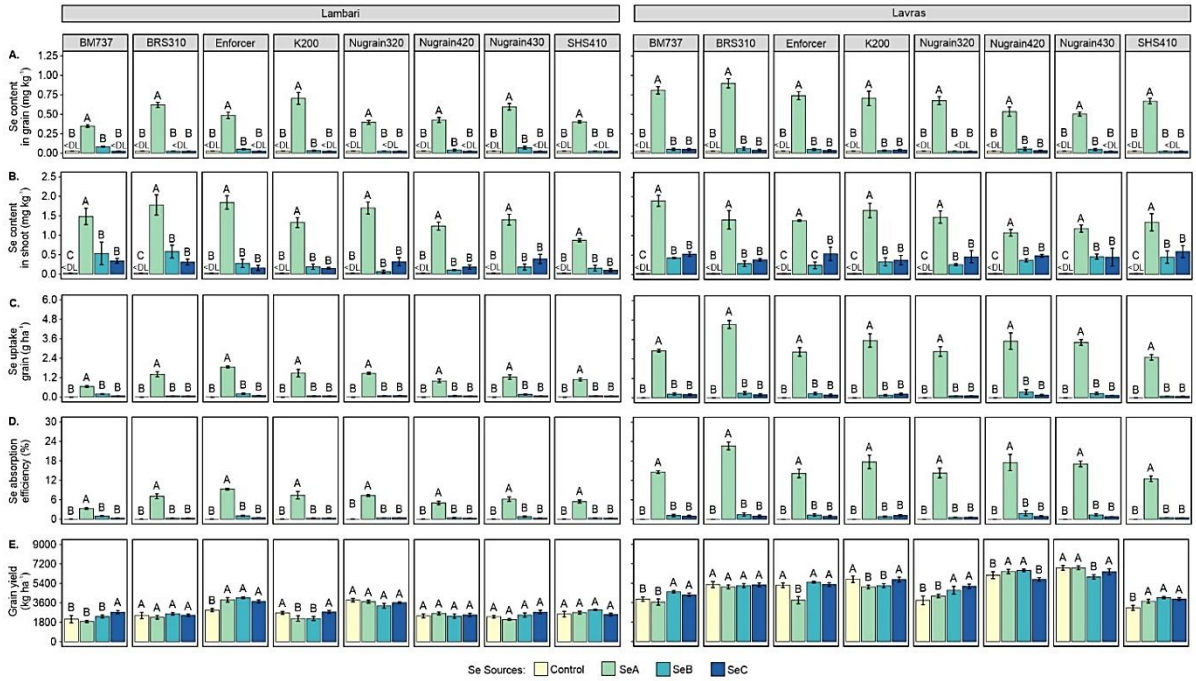

Fig. 4 – Field-grown conditions. Selenium content in grain (A), Se content in the shoot (B), Se accumulation in grain (C), Se uptake by grain (D), Se absorption efficiency (E), grain yield (F). The capital letters compare the Se doses in the same cultivar. Different letters indicate significant differences between treatments at a probability level of 5% ($p<0.05$) by test Scott-Knott. The bars show means, and the vertical error bars refer to the standard errors (n=4).

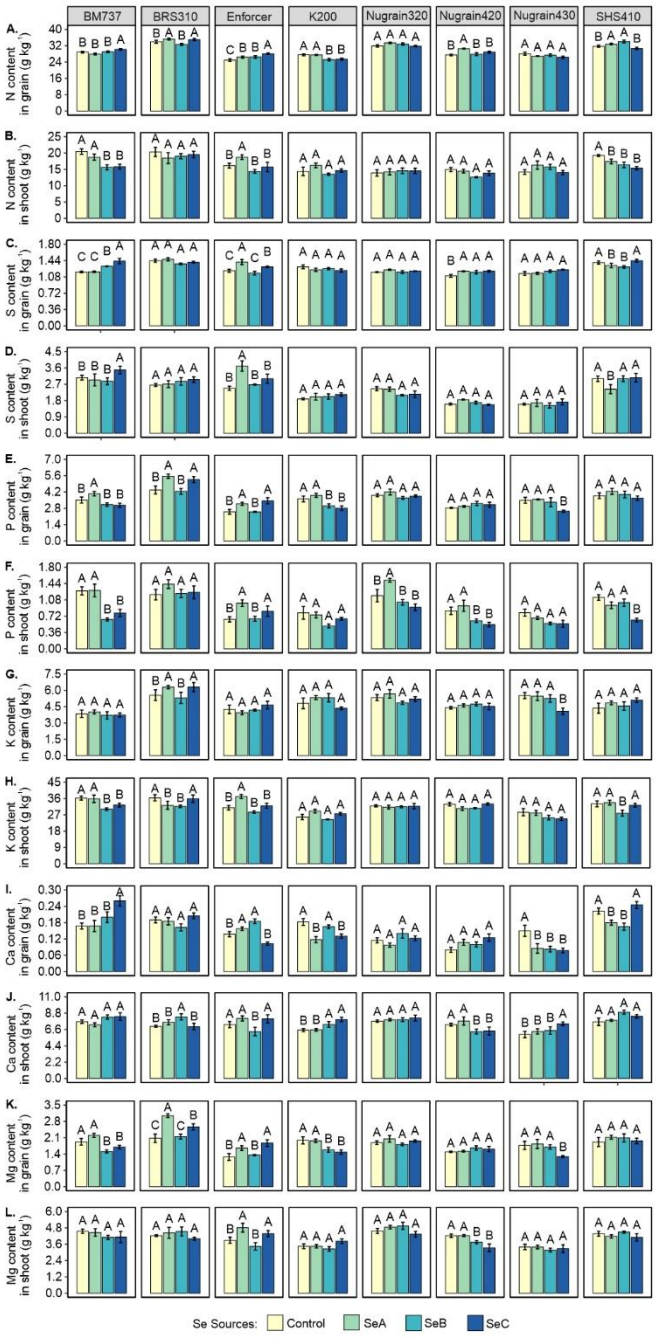

Fig. 5 – Greenhouse-grown conditions. The macronutrients contents in the grain and the shoot of the sorghum plants. The capital letters compare the Se source in the same cultivar. Different letters indicate significant differences between treatments at a probability level of 5% ($p<0.05$) by test Scott-Knott. The bars show means, and the vertical error bars refer to the standard errors (n=4).

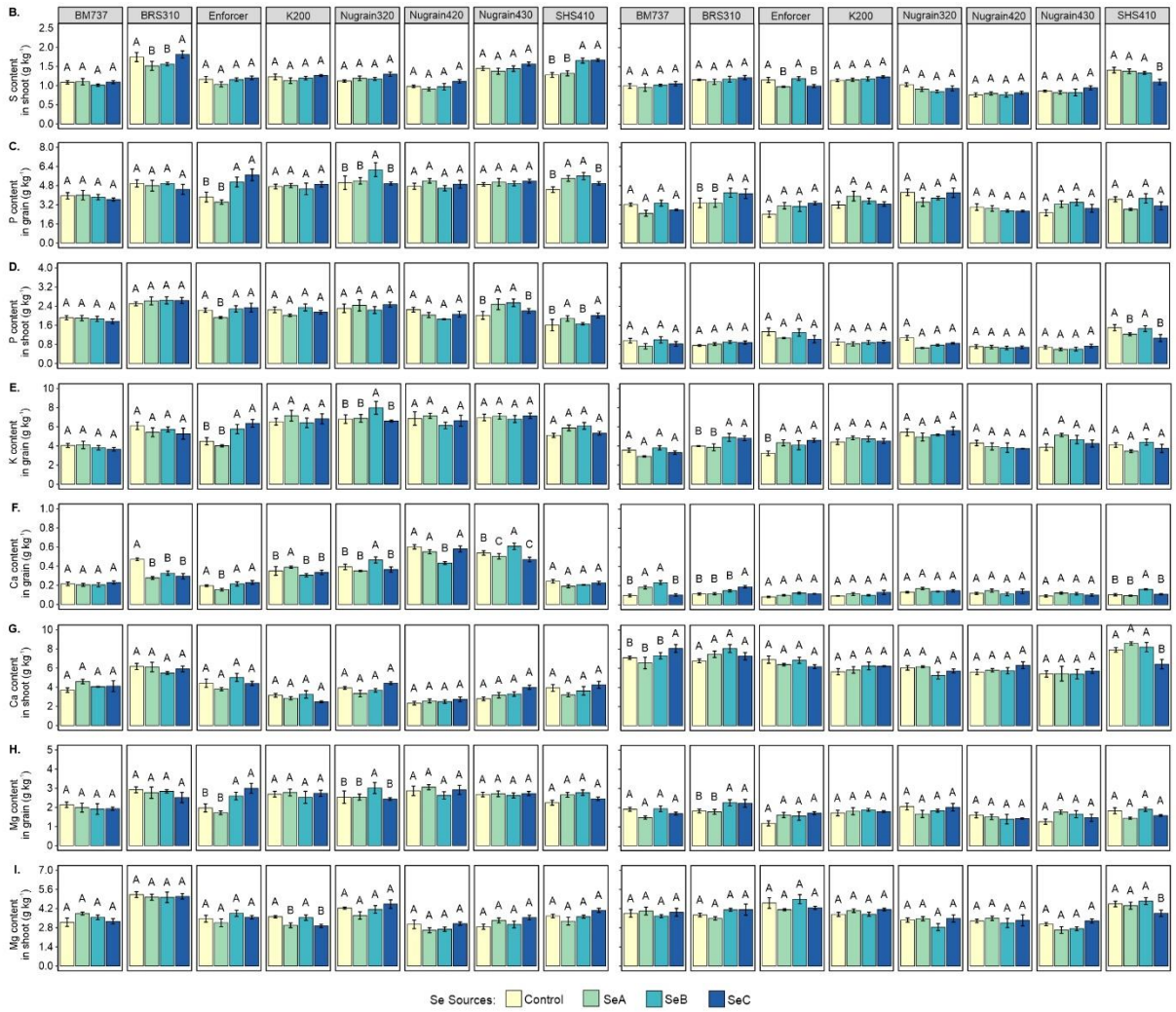

Fig. 6 – Field-grown conditions. The macronutrients contents in the grain and the shoot of the sorghum plants. The capital letters compare Se sources in the same cultivar. Different letters indicate significant differences between treatments at a probability level of 5% ($p<0.05$) by test Scott-Knott. The bars show means, and the vertical error bars refer to the standard errors (n=4).

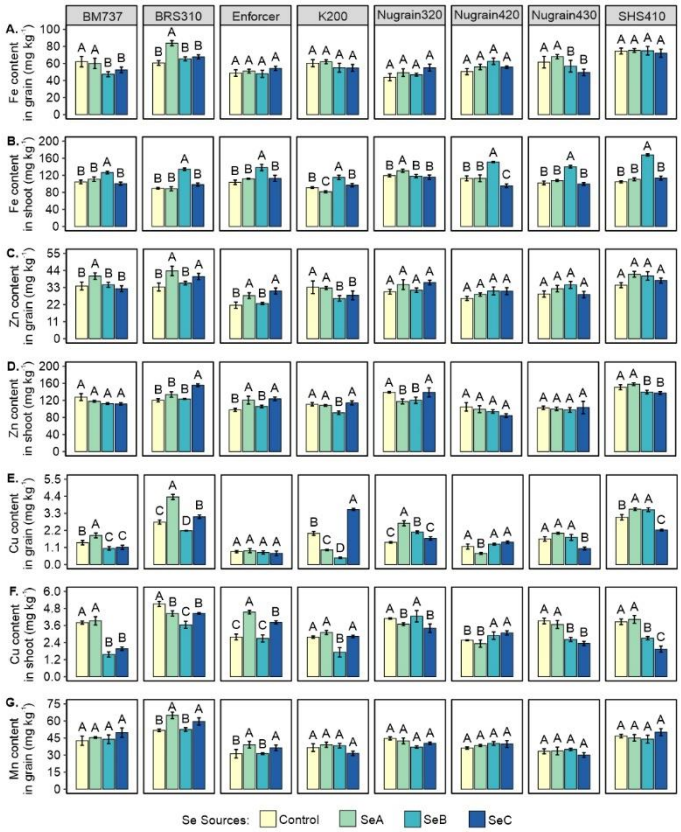

Fig. 7 – Greenhouse-grown conditions. The micronutrients contents in the grain and the shoot of the sorghum plants. The capital letters compare the Se in the same cultivar. Different letters indicate significant differences between treatments at a probability level of 5% ($p<0.05$) by test Scott-Knott. The bars show means, and the vertical error bars refer to the standard errors (n=4).

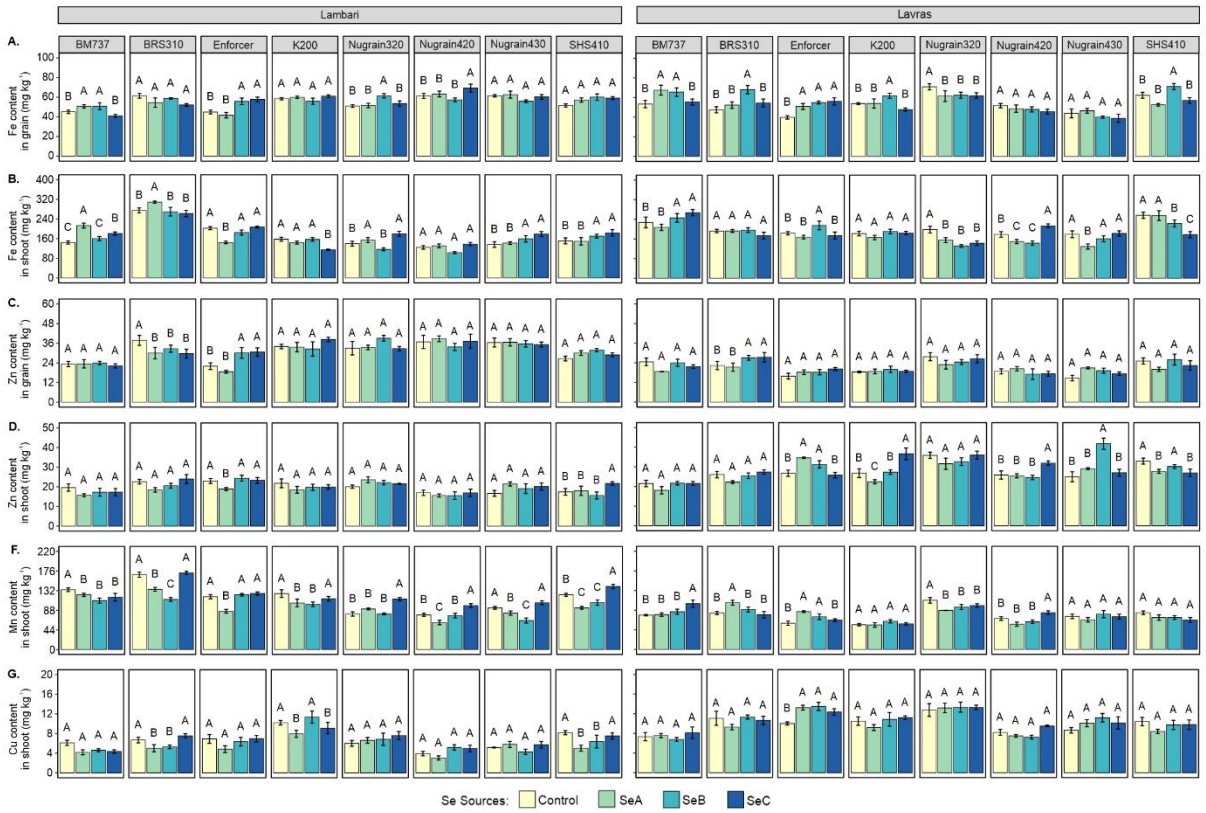

Fig. 8 – Field-grown conditions. The micronutrients contents in the grain and the shoot of the sorghum plants. The capital letters compare Se sources in the same cultivar. Different letters indicate significant differences between treatments at a probability level of 5% ($p<0.05$) by test Scott-Knott. The bars show means, and the vertical error bars refer to the standard errors (n=4).

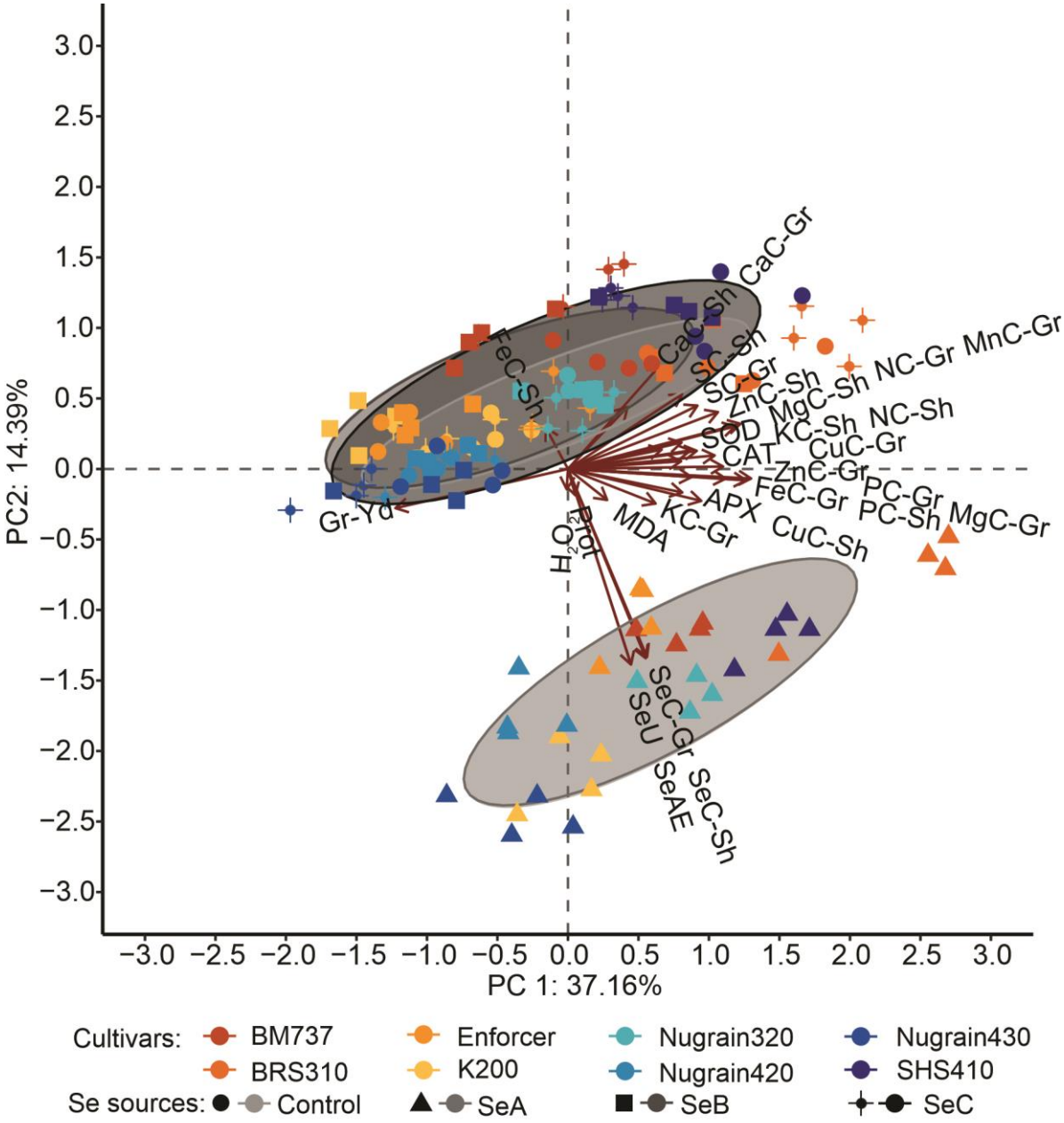

Fig. 9 - Principal component analysis in the greenhouse-grown. Abbreviations: Se content in grain (SeC-Gr) and in shoot (SeC-Sh); Se absorption efficiency (SeAE); Se uptake (SeU); lipid peroxidation (MDA); hydrogen peroxide (H_2O_2); catalase (CAT); ascorbate peroxidase (APX), superoxide dismutase (SOD), proteins in extract enzymatic (Prot); grain yield (Gr-Yd); N (NC-Gr), S (SC-Gr), P (PC-Gr), K (KC-Gr), Mg (MgC-Gr), Fe (FeC-Gr), Zn (ZnC-Gr), Mn (MnC-Gr), Cu (CuC-Gr) content in the grain; N (NC-Sh), S (SC-Sh), P (PC-Sh), K (KC-Sh), Mg (MgC-Sh), Ca (CaC-Sh); Fe (FeC-Sh); Zn (ZnC-Sh), Mn (MnC-Sh) and Cu (CuC-Sh) content in the shoot.

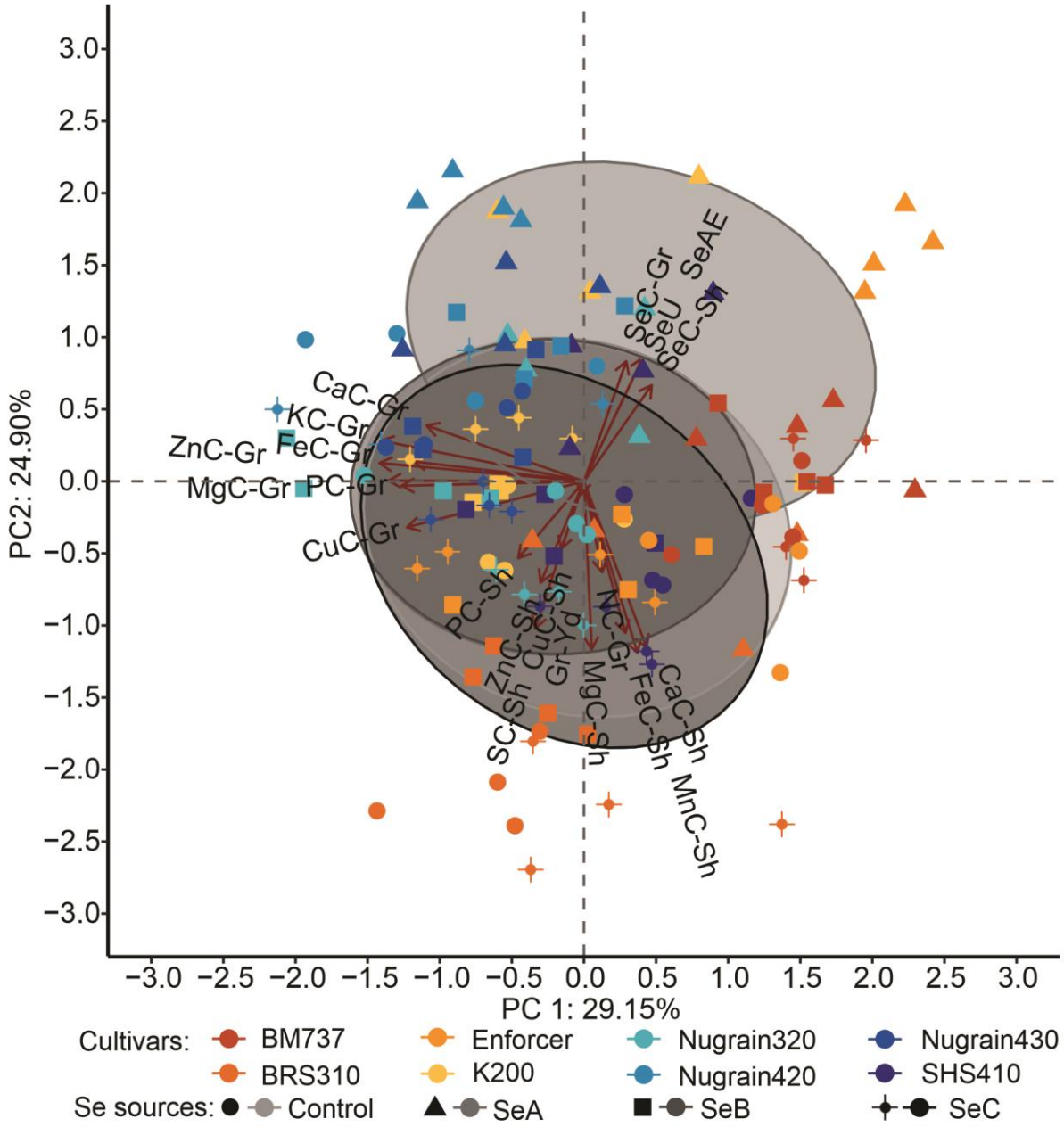

Fig. 10 - Principal component analysis in the field-grown sorghum at Lambari. Abbreviations: Se content in grain (SeC-Gr) and in shoot (SeC-Sh); Se absorption efficiency (SeAE); Se uptake (SeU); grain yield (Gr-Yd); N (NC-Gr), S (SC-Gr), P (PC-Gr), K (KC-Gr), Mg (MgC-Gr), Fe (FeC-Gr), Zn (ZnC-Gr), Mn (MnC-Gr), Cu (CuC-Gr) content in the grain; N (NC-Sh), S (SC-Sh), P (PC-Sh), K (KC-Sh), Mg (MgC-Sh), Ca (CaC-Sh); Fe (FeC-Sh); Zn (ZnC-Sh), Mn (MnC-Sh) and Cu (CuC-Sh) content in the shoot.

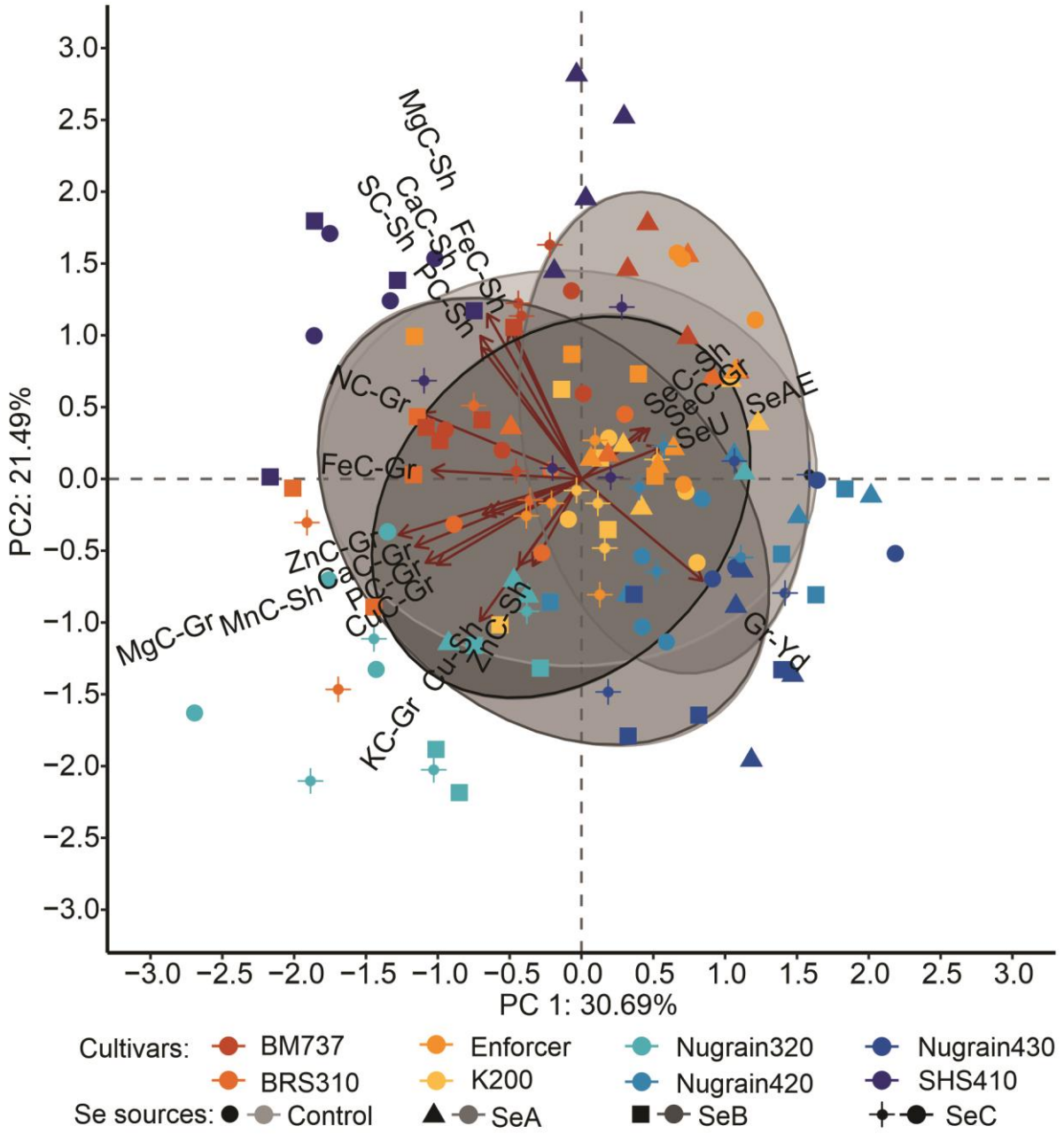

Fig. 11 - Principal component analysis in the field-grown sorghum at Lavras. Abbreviations: Se content in grain (SeC-Gr) and in shoot (SeC-Sh); Se absorption efficiency (SeAE); Se uptake (SeU); grain yield (Gr-Yd); N (NC-Gr), S (SC-Gr), P (PC-Gr), K (KC-Gr), Mg (MgC-Gr), Fe (FeC-Gr), Zn (ZnC-Gr), Mn (MnC-Gr), Cu (CuC-Gr) content in the grain; N (NC-Sh), S (SC-Sh), P (PC-Sh), K (KC-Sh), Mg (MgC-Sh), Ca (CaC-Sh); Fe (FeC-Sh); Zn (ZnC-Sh), Mn (MnC-Sh) and Cu (CuC-Sh) content in the shoot.

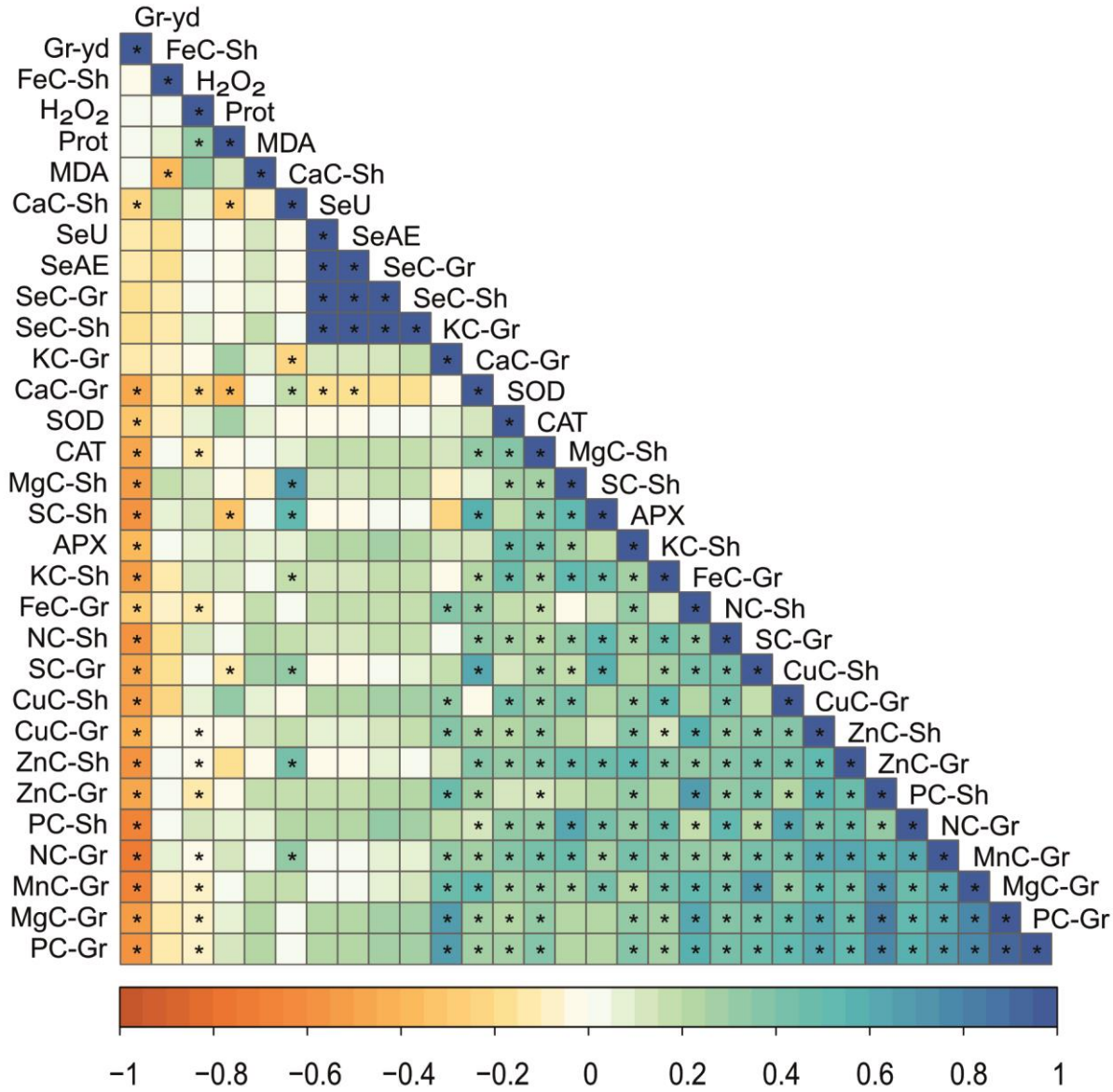

Fig. 12 - Heatmap showing the Pearson's correlation the significance of relationship identified with * was significant by F-test at $p \leq 0.05$ in the greenhouse-grown. Abbreviations: Se content in grain (SeC-Gr) and in shoot (SeC-Sh); Se absorption efficiency (SeAE); Se uptake (SeU); lipid peroxidation (MDA); hydrogen peroxide (H_2O_2); catalase (CAT); ascorbate peroxidase (APX), superoxide dismutase (SOD), proteins in extract enzymatic (Prot); grain yield (Gr-Yd); N (NC-Gr), S (SC-Gr), P (PC-Gr), K (KC-Gr), Mg (MgC-Gr), Fe (FeC-Gr), Zn (ZnC-Gr), Mn (MnC-Gr), Cu (CuC-Gr) content in the grain; N (NC-Sh), S (SC-Sh), P (PC-Sh), K (KC-Sh), Mg (MgC-Sh), Ca (CaC-Sh); Fe (FeC-Sh); Zn (ZnC-Sh), Mn (MnC-Sh) and Cu (CuC-Sh) content in the shoot.

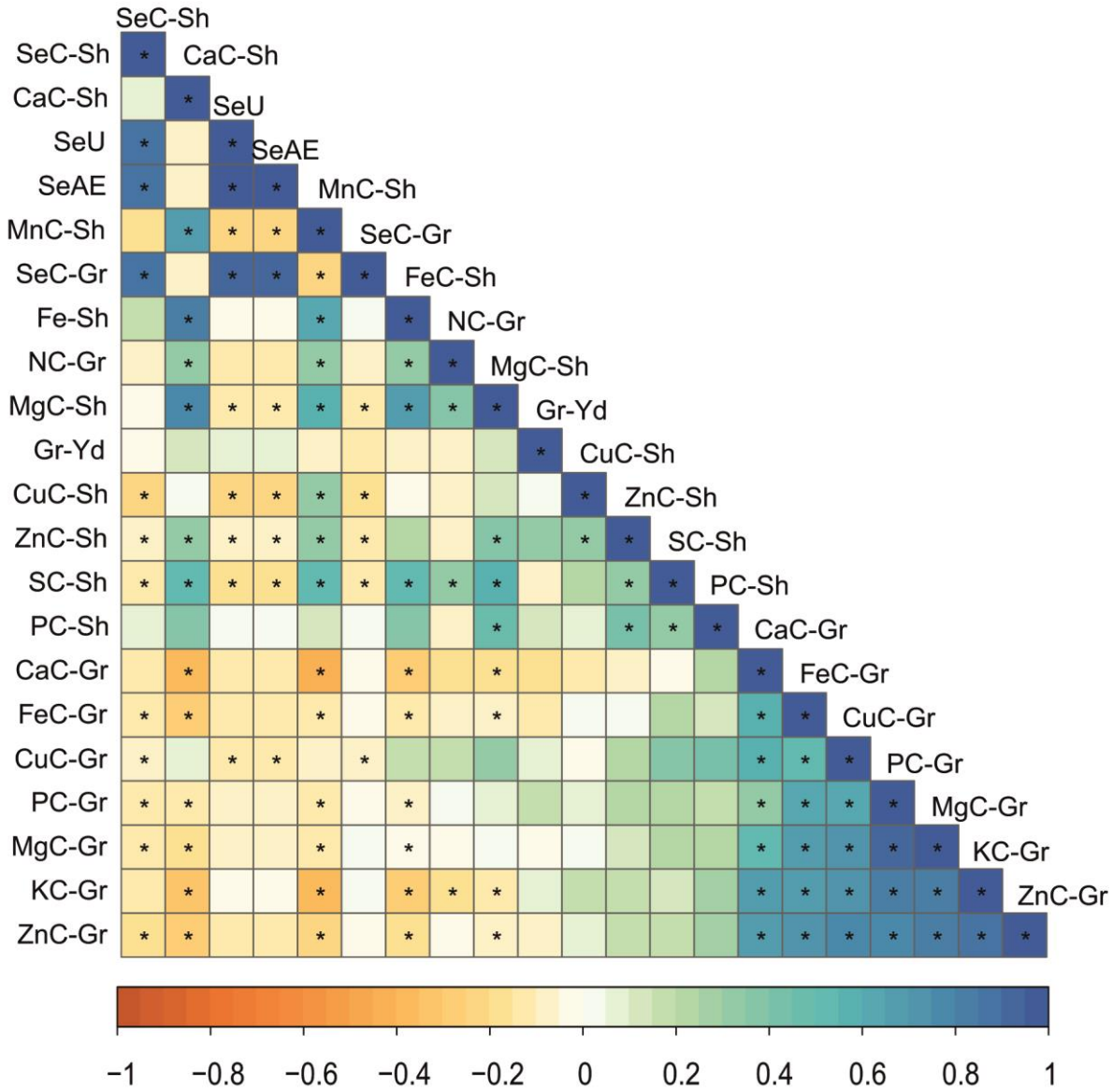

Fig. 13 - Heatmap showing the Pearson's correlation the significance of relationship identified with * was significant by F-test at $p \leq 0.05$ in the field-grown sorghum at Lambari. Abbreviations: Se content in grain (SeC-Gr) and in shoot (SeC-Sh); Se absorption efficiency (SeAE); Se uptake (SeU); grain yield (Gr-Yd); N (NC-Gr), S (SC-Gr), P (PC-Gr), K (KC-Gr), Mg (MgC-Gr), Fe (FeC-Gr), Zn (ZnC-Gr), Mn (MnC-Gr), Cu (CuC-Gr) content in the grain; N (NC-Sh), S (SC-Sh), P (PC-Sh), K (KC-Sh), Mg (MgC-Sh), Ca (CaC-Sh); Fe (FeC-Sh); Zn (ZnC-Sh), Mn (MnC-Sh) and Cu (CuC-Sh) content in the shoot.

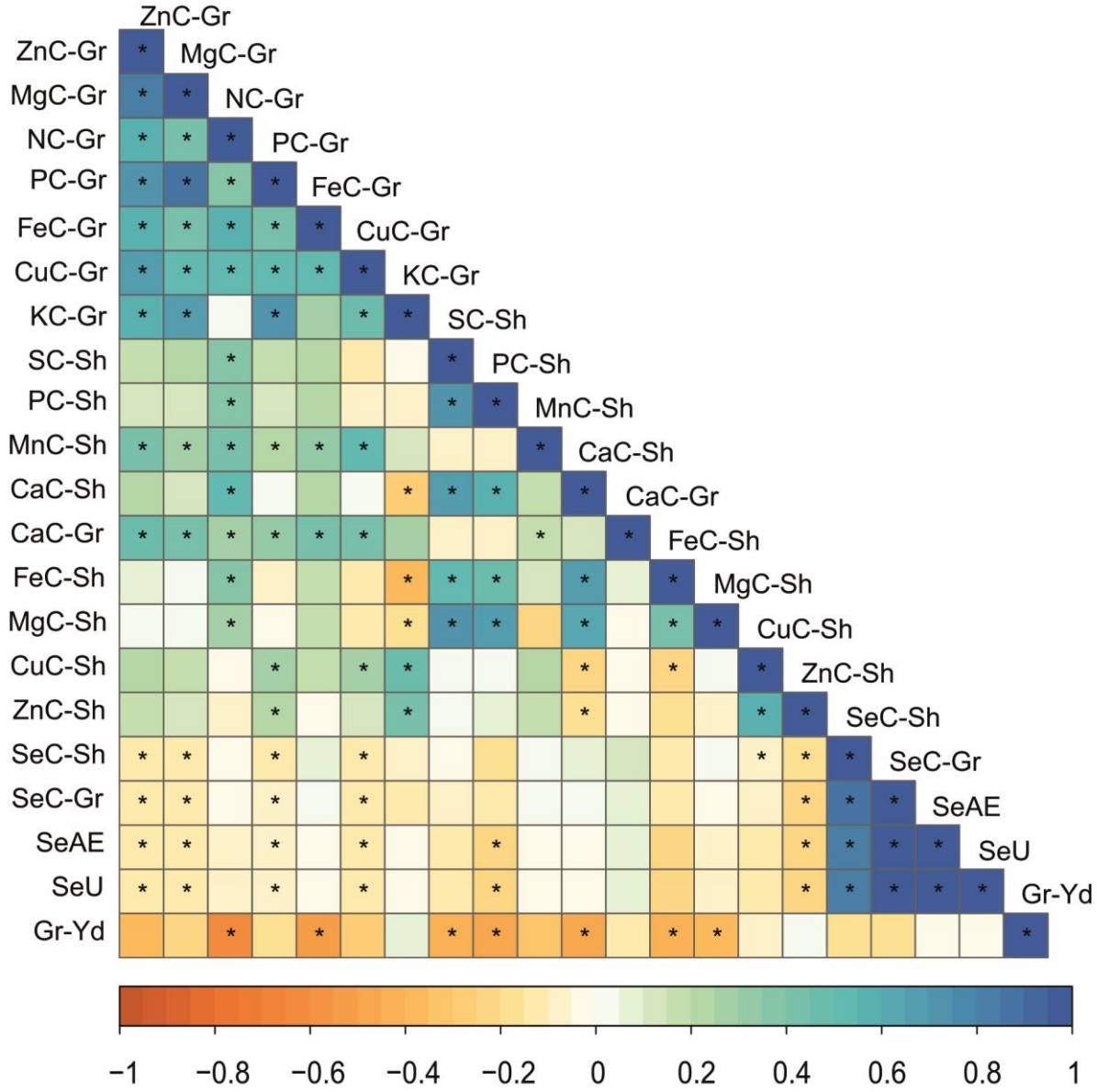

Fig. 14 - Heatmap showing the Pearson's correlation the significance of relationship identified with * was significant by F-test at $p \leq 0.05$ in the field-grown sorghum at Lavras. Abbreviations: Se content in grain (SeC-Gr) and in shoot (SeC-Sh); Se absorption efficiency (SeAE); Se uptake (SeU); grain yield (Gr-Yd); N (NC-Gr), S (SC-Gr), P (PC-Gr), K (KC-Gr), Mg (MgC-Gr), Fe (FeC-Gr), Zn (ZnC-Gr), Mn (MnC-Gr), Cu (CuC-Gr) content in the grain; N (NC-Sh), S (SC-Sh), P (PC-Sh), K (KC-Sh), Mg (MgC-Sh), Ca (CaC-Sh); Fe (FeC-Sh); Zn (ZnC-Sh), Mn (MnC-Sh) and Cu (CuC-Sh) content in the shoot.

Material Supplementary

Table S2. Chemical characterization and particle size distribution before sowing of the soil used in the greenhouse-grown and field-grown sorghum in Lambari e Lavras.

	Units	Method/ Extractant	Greenhouse	Lambari		Lavras	
				0-20cm	20-40cm	0-20cm	20-40cm
Sand	g kg^{-1}	Hydrometer	730	250	440	570	520
Silt	g kg^{-1}	Hydrometer	50	200	260	160	180
Clay	g kg^{-1}	Hydrometer	220	550	300	270	300
SOM	g kg^{-1}	$Na_2Cr_2O_7$ 4N + H_2SO_4 10N	6.8	4.82	4.08	2.04	1.54
pH		Water 1:2.5	5.1	5.7	5.6	5.8	60
Al	$cmol_c$ dm^{-3}	KCl (1 mol L^{-1})	0.45	0.18	0.24	0.14	0.09
Al+H	$cmol_c$ dm^{-3}	SMP	2.37	5.83	6.38	1.96	2.48
P	mg dm^{-3}	Mehlich-1	0.71	58.32	36.16	5.60	1.72
K	mg dm^{-3}	Mehlich-1	23.46	115.2	77.70	57,86	43.33
Ca	$cmol_c$ dm^{-3}	KCl (1 mol L^{-1})	0.19	4.84	2.53	3.12	2.50
Mg	$cmol_c$ dm^{-3}	KCl (1 mol L^{-1})	0.10	1.70	0.96	0.85	0.77
S	mg dm^{-3}	$Ca(H_2PO_4)_2$ + CH_3COOH	4.23	9.63	18.97	3.57	3.49
B	mg dm^{-3}	Hot water	0.07	0.14	0.10	0.22	0.12
Cu	mg dm^{-3}	Mehlich-1	0.10	1.88	1.81	1.06	0.76
Fe	mg dm^{-3}	Mehlich-1	47.33	32.19	23.73	61.86	45.11
Mn	mg dm^{-3}	Mehlich-1	2.44	8.88	5.20	30.39	20.33
Zn	mg dm^{-3}	Mehlich-1	0.28	0.85	0.54	1.76	0.70
Se	mg kg^{-1}	USEPA3051A	<DL	<DL	<DL	<DL	<DL

SOM – soil organic matter; DL – detection limit.

Table S3 – Lines used to determine the elements with ICP-OES and assessment of precision through the analysis of Peach Leaves SRM1547.

Element or Nutrient	λ (nm)	Certified[a] (mg kg^{-1})	Found[b] (mg kg^{-1})	% Recovery[c]
S	180.731	2000	1566±501	78
P	178.287	1371±82	1343±43	98
K	769.896	24330±380	25584±528	105
Ca	315.887	15590±160	16948±501	109
Mg	279.079	4320±150	4586±139	106
Fe	373.486	219.8±6.8	214±10,9	97
Cu	324.754	3.75±0.37	2.70±0.22	72
Zn	213.856	17.97±0.53	17.3±0.96	96
Mn	403.076	97.8±1.8	113±2.39	116

[a] Results for Peach Leaves SRM1547 represented as mean ± confidence interval, informative value. [b] Mean ± standard error of the mean. Average of ten determinations. [c] Average of ten determinations.

Table S4 - Analysis of variance of eight sorghum cultivars cultivated in greenhouse and in field conditions, fertilized with different Se sources.

Source of variation	F- test													
	Greenhouse				Lambari					Lavras				
	C	S	CxS	CV	B	C	S	CxS	CV	B	C	S	CxS	CV
H_2O_2	*	ns	**	16.63	---	---	---	---	---	---	---	---	---	---
MDA	**	**	**	13.14	---	---	---	---	---	---	---	---	---	---
SOD	**	*	*	12.21	---	---	---	---	---	---	---	---	---	---
CAT	**	**	**	16.69	---	---	---	---	---	---	---	---	---	---
APX	**	**	**	15.53	---	---	---	---	---	---	---	---	---	---
Protein	**	*	**	14.90	---	---	---	---	---	---	---	---	---	---
SeC-Gr	**	**	**	19.57	ns	**	**	**	27.69	ns	**	**	**	29.35
SeC-Sh	**	**	**	14.33	ns	**	**	**	44.82	ns	ns	**	ns	39.70
SeU	**	**	**	23.46	ns	**	**	**	34.78	ns	**	**	**	33.78
SeAE	**	**	**	23.75	ns	**	**	**		ns	**	**	**	33.78
Grain yield	**	**	**	8.21	ns	**	*	**	12.28	ns	**	**	**	8.72
Grain Weight	**	ns	ns	15.69	**	**	**	ns	8.15	ns	**	ns	ns	5.27
NC-Gr	**	*	**	3.45	ns	**	ns	ns	5.53	ns	**	ns	ns	4.60
SC-Gr	**	**	**	4.91	**	**	ns	ns	4.78	ns	**	**	ns	4.28
PC-Gr	**	**	**	11.57	ns	**	ns	**	13.21	ns	**	ns	*	16.85
KC-Gr	**	ns	*	12.85	*	**	ns	**	12.80	ns	**	ns	*	14.41
CaC-Gr	**	**	**	16.45	**	**	**	**	12.17	ns	**	**	**	20.23
MgC-Gr	**	**	**	12.53	*	**	ns	**	14.74	ns	**	ns	*	16.61
FeC-Gr	**	**	*	13.52	ns	**	ns	**	8.83	**	**	**	**	11.08
ZnC-Gr	**	**	*	13.02	**	**	ns	*	14.63	ns	**	ns	ns	17.88
CuC-Gr	**	**	**	12.47	**	**	ns	*	16.82	ns	**	**	**	25.76
MnC-Gr	**	*	*	12.10	**	**	**	ns	13.86	ns	**	ns	ns	15.67
NC-Sh	**	**	*	10.85	**	**	*	ns	17.46	ns	**	**	**	12.43
SC-Sh	**	ns	**	13.60	**	**	**	*	9.34	ns	**	ns	**	10.29
PC-Sh	**	**	**	18.55	ns	**	ns	ns	12.12	ns	**	**	ns	20.00
KC-Sh	**	**	**	8.12	ns	**	*	ns	11.73	ns	**	ns	ns	9.73
CaC-Sh	**	*	**	9.79	*	**	ns	**	14.34	ns	**	ns	**	10.79
MgC-Sh	**	*	*	10.77	ns	**	ns	*	11.28	ns	**	ns	ns	12.21
FeC-Sh	**	**	**	7.52	ns	**	**	**	10.77	ns	**	**	**	12.32
ZnC-Sh	**	**	**	9.88	**	**	ns	*	14.58	**	**	**	**	11.95
CuC-Sh	**	**	**	12.38	**	**	**	**	19.80	**	**	*	**	12.49
MnC-Sh	**	ns	ns	11.31	*	**	**	**	8.73	ns	**	ns	**	12.52

ns – not significant by F-test; * - significant by F-test at p<0,05; ** - significant by F-test at p<0,01; CV – coefficient of variation (%).

Degrees of freedom, greenhouse: cultivars (C) – 7; doses (D) – 3; C x D – 21; residue – 96

Degrees of freedom, field: block (B) – 3; cultivars (C) – 7; doses (D) – 3; C x D – 21; residue – 93

Abbreviations: Se content in grain (SeC-Gr) and in shoot (SeC-Sh); Se absorption efficiency (SeAE); Se uptake (SeU); lipid peroxidation (MDA); hydrogen peroxide (H_2O_2); catalase (CAT); ascorbate peroxidase (APX), superoxide dismutase (SOD); N (NC-Gr), S (SC-Gr), P (PC-Gr), K (KC-Gr), Mg (MgC-Gr), Fe (FeC-Gr), Zn (ZnC-Gr), Mn (MnC-Gr), Cu (CuC-Gr) content in the grain; N (NC-Sh), S (SC-Sh), P (PC-Sh), K (KC-Sh), Mg (MgC-Sh), Ca (CaC-Sh); Fe (FeC-Sh); Zn (ZnC-Sh), Mn (MnC-Sh) and Cu (CuC-Sh) content in the shoot.

Table S5 - Joint-variance analysis of eight sorghum cultivars in field-grown sorghum (Lambari and Lavras) fertilized with different Se sources.

Source of variation	FMax	F-test									
		Block (B)	Cultivar (C)	Source (S)	Local (L)	BxL	CxS	LxC	LxS	LxCxS	CV (%)
SeC-Gr	2.01	ns	**	**	**	ns	**	**	**	**	29.07
SeC-Sh	1.03	ns	**	**	*	ns	**	**	**	*	42.09
SeU	5.91	ns	**	**	**	ns	**	**	**	**	36.91
SeAE	5.90	ns	**	**	**	ns	**	**	**	**	36.90
Grain yield	1.734	ns	**	**	**	ns	**	**	ns	**	10.06
Grain Weight	1.503	**	**	**	**	**	ns	**	*	ns	6.57
NC-Gr	1.895	ns	**	ns	**	ns	*	**	ns	ns	5.16
SC-Gr	2.232	**	**	ns	**	*	ns	**	**	ns	4.66
PC-Gr	1.303	ns	**	*	**	ns	**	**	ns	**	14.76
KC-Gr	1.549	*	**	ns	**	ns	**	**	ns	*	13.55
CaC-Gr	2.731	*	**	ns	**	*	**	**	**	**	14.75
MgC-Gr	1.752	ns	**	ns	**	*	**	**	ns	**	15.64
FeC-Gr	1.499	*	**	**	*	**	**	**	*	**	9.99
ZnC-Gr	1.485	**	**	ns	**	ns	*	**	ns	*	16.01
CuC-Gr	2.148	**	**	**	**	**	**	**	ns	ns	19.92
MnC-Gr	2.076	**	**	*	**	*	ns	**	*	ns	14.79
NC-Sh	2.547	*	**	**	**	**	ns	**	*	ns	15.50
SC-Sh	1.233	ns	**	**	**	**	ns	**	**	**	9.78
PC-Sh	2.085	ns	**	ns	**	ns	ns	**	ns	*	14.69
KC-Sh	1.156	ns	**	*	**	ns	ns	**	ns	ns	10.61
CaC-Sh	1.624	*	**	ns	**	ns	ns	**	ns	**	12.20
MgC-Sh	1.151	ns	**	ns	*	ns	ns	**	ns	*	11.98
FeC-Sh	1.584	ns	**	*	**	ns	**	**	**	**	11.66
ZnC-Sh	1.234	**	**	**	**	**	**	**	ns	**	12.66
CuC-Sh	1.059	**	**	**	**	**	**	**	*	**	15.25
MnC-Sh	1.041	**	**	**	**	ns	**	**	**	**	10.30

ns – not significant by F-test; * - significant by F-test at p<0,05; ** - significant by F-test at p<0,01; CV – coefficient of variation (%).

Degrees of freedom field: block (B) – 3; cultivars (C) – 7; doses (D) – 3; Local (L) – 1; B x L – 3; C x D – 21; L x C – 7; L x D – 3; L x C x D – 21; residue – 186.

Abbreviations: Se content in grain (SeC-Gr) and in shoot (SeC-Sh); Se absorption efficiency (SeAE); Se uptake (SeU); N (NC-Gr), S (SC-Gr), P (PC-Gr), K (KC-Gr), Mg (MgC-Gr), Fe (FeC-Gr), Zn (ZnC-Gr), Mn (MnC-Gr), Cu (CuC-Gr) content in the grain; N (NC-Sh), S (SC-Sh), P (PC-Sh), K (KC-Sh), Mg (MgC-Sh), Ca (CaC-Sh); Fe (FeC-Sh); Zn (ZnC-Sh), Mn (MnC-Sh) and Cu (CuC-Sh) content in the shoot.

CONSIDERAÇÕES FINAIS E PERSPECTIVAS FUTURAS

A pesquisa já comprovou a essencialidade do Se para humanos e animais, bem como sua atuação no sistema de defesa oxidativa. Embora não tenha sido comprovada a essencialidade do Se para plantas, há estudos que comprovaram sua eficiência na mitigação de estresses abióticos e na biofortificação de várias plantas. Quando consideramos a cadeia alimentar, observa-se que a ingestão média diária de Se por humanos e animais é limitada devido aos solos de várias regiões do mundo apresentarem baixos teores desse elemento. Sendo assim, é necessário aplicar técnicas para suprir os baixos teores de Se nos alimentos, e consequentemente, aumentar sua quantidade e disponibilidade por toda a cadeia alimentar, combatendo a desnutrição deste mineral.

A biofortificação agronômica é uma técnica que tem sua eficiência comprovada para suprir a deficiência de minerais. Estudos avaliando os efeitos da biofortificação com Se têm sido reportados em várias culturas de interesse agronômico como frutas, vegetais e cereais. Essa técnica pode ser facilmente empregada pelos produtores por serem de fácil acesso e de aplicação simples. No entanto, ainda há muito a ser feito no tocante à divulgação dos benefícios dessa prática em todo o meio agrícola.

Os cereais são de grande interesse para a pesquisa e, neste cenário, destaque maior tem sido dado ao arroz e ao trigo. No entanto, estudos com o sorgo são incipientes. O sorgo é uma espécie que tem ganhado destaque no cenário atual devido aos altos preços alcançados pelo milho. O sorgo pode ser um substituto para o milho em formulações de rações para animais como aves, suínos e bovinos. Na alimentação humana, o sorgo pode substituir o trigo em receitas tradicionais permitindo uma redução nos custos dos alimentos e também o consumo por pessoas celíacas, devido à ausência de glúten em sua composição. Neste contexto, observa-se que a biofortificação do sorgo com Se é um nicho que precisa ser explorado.

Este estudo foi pioneiro, em condições de solos e cultivares brasileiros, em relação à biofortificação com Se em sorgo. Observou-se neste estudo que a aplicação de Se no solo ou via foliar na forma de selenato de sódio aumentou os teores de Se nos grãos de sorgo, o que faz dessa uma espécie viável para a biofortificação agronômica. A estratégia envolvendo a aplicação foliar do Se é mais indicada para as plantas de sorgo, uma vez que a adubação foliar é uma técnica que é empregada com frequência e facilidade, requer uma menor quantidade de Se na solução a ser aplicada e promoveu incrementos satisfatórios nos teores de Se nos grãos de sorgo.

A aplicação de fontes orgânicas de Se promoveu menores teores de Se quando comparadas ao selenato aplicado via solo e teores de Se abaixo do limite de detecção e quantificação quando aplicado via foliar. No entanto, por serem fontes de Se que ainda não haviam sido estudadas, sendo estas inovadoras no processo de biofortificação, novos estudos precisam ser feitos. Para validação de técnicas de aplicação das fontes de Se orgânico no solo, são necessários novos estudos avaliando a influência do tipo de solo, pH, potencial de oxidação, capacidade de sorção e dessorção, matéria orgânica e processos biológicos sobre estas fontes. Também a possibilidade de revestimento de grânulos de outros fertilizantes com essas fontes, complexação com algum polímero que favoreça a absorção pelas plantas e menor retenção no solo são fatores a serem avaliados.

Em relação à aplicação foliar das fontes orgânicas de Se, deve-se considerar a possibilidade de ter havido uma interação entre os compostos com Se e o surfactante utilizado, justificando assim a baixa absorção de Se pelas plantas quando da aplicação dessas fontes de Se. Depreende-se, pois, que novos estudos precisam ser feitos verificando a interação entre as fontes de Se e outros produtos utilizados na pulverização foliar para promover a melhor absorção do nutriente contido na solução aplicada. Também é necessário avaliar a possibilidade da aplicação conjunta do Se com outros produtos químicos aplicados na cultura do sorgo, a fim de otimizar o trabalho e reduzir o número de pulverizações, gerando redução de custos com mão-de-obra.

Além do sorgo granelífero, há outros tipos de sorgo, como o sorgo forrageiro e sorgo para pastejo. Sendo assim, é necessário e interessante avaliar, ainda, o potencial desses tipos de sorgo para a biofortificação com Se. Ao considerar todos os tipos de sorgo, também é necessário avaliar outras cultivares, melhor estádio fenológico para a biofortificação, número de aplicações de Se, interação com outros minerais, efeitos no teor de proteínas, carboidratos, no sistema antioxidante, e na qualidade da silagem produzida.

Embora estudos preliminares com biofortificação com Se sejam feitos em ambiente protegido, essa prática minimiza os efeitos do ambiente. O sorgo pode ser cultivado em várias regiões e, assim sendo, estudos em campo se justificam para determinar a dose de Se ideal e o efeito sobre a produção de grão e de biomassa vegetal nos mais variados ambientes de cultivo. Visto isso, além da avaliação agronômica é de grande importância a avaliação de parâmetros fisiológicos e bioquímicos, pois estes podem justificar os ganhos agronômicos e nutricionais.