GUILHERME AMARAL DE SOUZA

BIOFORTIFICAÇÃO DA CULTURA DO TRIGO COM ZINCO, SELÊNIO E FERRO:
EXPLORANDO O GERMOPLASMA BRASILEIRO

LAVRAS – MG

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

Orientadores

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APROVADA em 09 de Agosto de 2013.

Dra. Ana Rosa Ribeiro Bastos	UFLA
Dr. André Rodrigues dos Reis	Waseda University
Dra. Cleide Aparecida de Abreu	IAC
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Orientadores

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Sonhem...

Sonhem alto...

Mas lembrem-se que...

O que torna irrealizável um sonho, não é o sonho em si;

Mas sim, a inércia de quem sonha.

Alfredo Scheid Lopes

RESUMO

Nos últimos anos, há crescente preocupação de pesquisadores quanto à qualidade nutricional dos produtos agrícolas colhidos, em decorrência do incremento da produção de grãos e diminuição dos teores de nutrientes e outros elementos nos mesmos. Como consequência, observa-se uma baixa qualidade nutricional dos produtos que compõem a dieta básica da população, principalmente em países em desenvolvimento. Diante desse cenário, justifica-se o interesse pelos estudos da biofortificação, pois a desnutrição e a deficiência de micronutrientes são dois dos maiores desafios agrícolas do século. O presente trabalho foi dividido em dois artigos, o primeiro trabalhou-se com "seedlings" de 20 acessos de trigo e, cujos objetivos foram: (i) avaliar os efeitos da aplicação de Fe, Zn e Se, no crescimento e acúmulo de nutrientes; (ii) estudar os efeitos dos tratamentos e suas interações com os demais nutrientes e (iii) definir potenciais linhagens a serem utilizadas para biofortificação agronômica. Já no segundo artigo, trabalhou-se com plantas de trigo, 20 acessos, até a produção de grãos e objetivou-se: (i) avaliar os efeitos das aplicações de Zn e Zn+Se, nos parâmetros agronômicos e teores de nutrientes, (ii) avaliar os efeitos dos tratamentos e suas interações com outros nutrientes em parte aérea e grãos e, (iii) com base nos resultados anteriores, definir quais os acessos são mais indicados para estudos de campo, visando a biofortificação. Como resultados, de forma geral, verificou-se que o germoplasma brasileiro mostrou-se diversificado e com potencial para o melhoramento genético e agronômico, para o enriquecimento com esses elementos. Os teores dos elementos aumentaram na parte aérea e grãos de trigo, mas vale ressaltar que há diferentes respostas dos acessos, devido à sua genética. Ademais, tanto em "seedlings" como em grãos, a aplicação de selenito mostrou-se mais restritiva ao crescimento e desenvolvimento desses germoplasmas de trigo que a de selenato, indicando que, para a biofortificação da cultura do trigo, o melhor seria a utilização de fontes ligadas ao selenato. Além disso, para o primeiro trabalho, verificou-se que as plantas foram mais susceptíveis quando submetidas a doses de 150 μM de Fe, no entanto incrementos foram observados em todos os acessos tratados com Zn. Como recomendação para biofortificação com Fe são indicados os acessos: EMB 14; EMB 34; EMB 38 e BRS 264; para Zn os acessos mais recomendados são: EMB11; EMB 20; EMB 38 e BRS 264; e para Se os acesso indicados seriam: EMB 10; EMB 14; EMB 38 e BRS 264. Para o segundo experimento, considerando-se os parâmetros agronômicos, acúmulo de nutrientes e a interação entre eles, os acessos recomendados seriam: BRS 207; BRS 254; BRS 264; EMB 19 e EMB 33 para biofortificação com Zn e os acessos: Brilhante, BRS 264; Supera EMB 7; EMB 20; EMB 26; EMB 30 e EMB 33 para Zn+Se.

Entretanto, ressalta-se que cada espécie apresenta uma resposta a determinado nutriente e/ou elemento, experimentos envolvendo outras culturas e espécies que compõem a dieta de populações são recomendados para desenvolvimento dessa técnica e amenização de problemas relacionados à segurança alimentar.

Palavras-chave: Micronutrientes. Segurança alimentar. Variabilidade genética. Selenato. Selenito.

ABSTRACT

In recent years, there is a growing concern about the nutritional quality of agricultural harvests, because selected approaches for grain production increase have resulted in a decrease of nutrients levels in many crops. As a consequence, there is a low nutritional quality of the products that are staple diet compounds of the population, especially in developing countries. This scenario justifies the current global interest on biofortification studies, as malnutrition and micronutrient deficiency are two of the greatest challenges of the century. This study was divided into two experiments, the first worked with 20 wheat accessions in seedlings stage and whose objectives were: (i) evaluate the effects of Fe, Zn and Se applications on growth and nutrient concentration; (ii) to study the effects of treatments and their interactions with other nutrients and; (iii) define potential accessions to be used for agronomic biofortification. In the second experiment we worked with wheat plants, 20 accessions, until the mature grain and aimed: (i) to evaluate the effects of Zn and Zn+Se addition in the agronomic parameters and nutrient accumulation in shoot and grain; (ii) to study treatment effects and their interactions with other nutrients in shoot and grain, and; (iii) based in the previous results, define which access is most suitable for field studies aimed at biofortification. As a result, in general, it was found that the Brazilian germplasm showed to be diverse and presented a great potential for genetic and agronomic enrichment with these elements. The content of the elements increased in wheat shoots and grains, but it is noteworthy that there are different responses of access due to their genotypic variation. In addition, for both seedlings and grains, the application of selenite was more restrictive to wheat growth and development than selenate. This result indicated that for biofortification of wheat crop it would be best to use selenate sources. Furthermore, for the first work, we found that wheat seedlings in general were less tolerable to high Fe exposure (150 μM), but benefits from increased level of Zn supply. In consideration of the nutrient concentration, plant growth and the interaction with other nutrients for the potential of increasing nutrient contents in grains among these wheat lines, EMB 14, EMB 34, EMB 38 and BRS 264 appeared to be appropriate for a biofortification program with Fe; many lines such as EMB 11, EMB 20, EMB 38 and BRS 264 for Zn; and EMB 10, EMB 14, EMB 38 and BRS 264 for Se. For the second experiment, taking into consideration the agronomic parameters, nutrient content, and potential of increasing nutrients in these wheat lines we indicate the accessions BRS 207; BRS 254; BRS 264; EMB 19 and EMB 33 for biofortification with Zn; many lines such as Brilhante; BRS 264; EMB 7; EMB 26 and EMB 30 appeared to be appropriate for $Zn+SeO_4$; and Brilhante; BRS 254; Supera; EMB 20 and EMB 33 for $Zn+SeO_3$. However, as each species has a specific response to nutrient and / or element, experiments involving other cultures and species that are staple food are recommended for developing this technique in order to overcome current issues related to food safety.

Keywords: Micronutrients. Food security. Genetic variability. Selenate. Selenite.

SUMÁRIO

PRIMEIRA PARTE

1 INTRODUÇÃO

A crescente produção de alimentos para satisfazer o consumo mundial fez com que a busca por produtividade elevada resultasse, em alguns casos, na perda de qualidade nutricional, principalmente após a Revolução Verde. Portanto, surge um novo desafio para a agricultura que consiste em melhorar a qualidade nutricional dos alimentos visando à nutrição humana, principalmente, quanto aos micronutrientes, ferro (Fe), zinco (Zn) e selênio (Se). Embora a produção de alimentos tenha acompanhado o crescimento populacional, problemas de deficiências nutricionais atingem grande parte da população mundial, especialmente mulheres grávidas, adolescentes e crianças.

Estima-se que, aproximadamente, um terço da população sofra com algum risco de deficiência de Fe e que, quase a mesma proporção, pode ser deficiente em Zn. Segundo a Organização da Nações Unidas, além das supracitadas, as deficiências de iodo (I), Se e vitamina A são as que causam maior preocupação em países em desenvolvimento. A principal razão é que, nesses países, a maioria das pessoas possuem uma dieta composta por alimentos de origem vegetal. Além disso, a biodisponibilidade dos minerais nos vegetais é, geralmente, baixa quando comparadas aos alimentos de origem animal. Desse modo, surge a necessidade de novas estratégias para promover aumentos nos teores e/ou biodisponibilidade de micronutrientes em alimentos. Aumentar os teores de nutrientes em culturas como arroz, trigo, milho, soja e feijão seria um grande passo para a solução do problema, já que essas culturas são as principais fontes desses nutrientes em muitas populações que se encontram sob algum tipo de risco de deficiência nutricional.

Nutrição de plantas e saúde humana são temas de elevada interface e não devem ser analisados isoladamente. A grande maioria dos elementos comprovadamente essenciais aos homens e aos animais também desempenha funções importantes no metabolismo vegetal e, em muitos casos, seus mecanismos de ação são similares. A escolha desse tema se deve ao fato de que pesquisadores em Ciência do Solo, aliados a outras ciências, podem e devem produzir alimentos com qualidade e em quantidade, por meio de novas técnicas, mantendo a sustentabilidade do solo e suprindo as demandas da sociedade.

Nos últimos 100 anos, a agricultura brasileira apresentou grande desenvolvimento, obtendo expressivos aumentos em produtividade. Tal fato, só foi possível devido às inovações tecnológicas, as pesquisas e a difusão das mesmas. Sem desmerecer outros fatores, as pesquisas em fertilidade do solo, principalmente as de uso eficiente de corretivos e fertilizantes, se destacam entre as mais importantes. No entanto, solos do Brasil possuem baixa fertilidade natural, decorrente da gênese do solo e alto grau de intemperismo que ocorre nas regiões tropicais. Como consequência, reporta-se a baixa disponibilidade de nutrientes (e.g. nitrogênio, fósforo, potássio, cálcio, magnésio, boro e Zn) e, portanto, sérias limitações à produção de alimentos nessas áreas. Apenas como exemplo, o uso adequado de corretivos e fertilizantes correspondem a um aumento de 50% na produção e produtividade. Por isso, intervenções no sistema, por meio do manejo da fertilidade do solo se faz necessário para o adequado suprimento das plantas, com esses nutrientes, e a consequente garantia de elevadas produtividades. Adicionalmente, a adição de fertilizantes pode aumentar os teores de nutrientes nas plantas e, portanto adicionar quantidades maiores dos mesmos na cadeia alimentar.

A cultura do trigo possui grande importância nas dietas. Estima-se que, cerca de 30% da população mundial, alimenta-se com algum produto derivado dessa cultura e, portanto, estudos que a envolvam são fundamentais para o desenvolvimento agrícola e sustentável de países como o Brasil. Ressalta-se, que mesmo importando quase 50% do trigo, o governo brasileiro tem estimulado pesquisas e incentivando produtores a aumentarem as áreas cultivadas visando à autossuficiência do país com esse grão. As interações entre micronutrientes podem afetar a absorção e a biodisponibilidade, por uma série de fatores. Os elementos em estudo, Se e Zn, não pertencem ao mesmo grupo periódico e nem competem pela absorção das plantas, mas será que há interação entre eles? Haverá efeito sinérgico para absorção de plantas? É possível biofortificar essas culturas, com esses elementos?

Sabe-se que pesquisas envolvendo variação genética de espécies cultivadas e biofortificação ainda são escassas e diante disso, para tentar responder a algumas dessas questões, objetivou-se, neste trabalho], avaliar o efeito de Zn e Se, bem como a interação entre esses elementos, em germoplasmas brasileiros de trigo.

2 REFERENCIAL TEÓRICO

2.1 Por que biofortificar plantas?

As plantas são o começo da cadeia alimentar e aumentos na absorção de nutrientes e seus acúmulos no tecido vegetal promovem melhorias nas dietas animais e humanas (PALMGREN et al., 2008). A dieta considerada adequada para seres humanos é composta por, pelo menos, 22 nutrientes, sendo a maior parte desses fornecida por vegetais, principalmente cereais que, se produzidos em áreas deficientes, produzem grãos com teores inadequados e/ou desbalanceados para a ingestão humana ou animal (ALLOWAY, 2009; WHITE; BROADLEY, 2005, 2009). Por si só, essa seria uma boa razão para intensificar os estudos de biofortificação, seja ela genética ou agronômica.

A produção sustentável de alimentos seguros e nutritivos é um objetivo da agricultura moderna. Contudo, nos últimos anos, esforços se concentraram em aumentar a produtividade das culturas. Porém, a qualidade das mesmas foi deixada de lado e a concentração de minerais tornou-se relevante, pois grande parte da população mundial sofre com a desnutrição e deficiência de ferro (Fe), zinco (Zn), selênio (Se), iodo (I) e vitamina A (WELCH; GRAHAM, 2002; WHITE; BROADLEY, 2009; WORLD HEALTH ORGANIZATION - WHO, 2002). Zhang et al. (2012) afirmam que o aumento em produtividade, sem qualidade nutricional, pode agravar a desnutrição. A explicação seria a baixa concentração de determinados nutrientes no grande volume produzido (GARVIN; WELCH; FINLEY, 2006; ZHANG et al., 2012). Para exemplificar, pela Figura 1, apresentam-se os efeitos do aumento de produtividade e a diminuição nos teores de Fe e Zn, em plantas de trigo e a variação nos teores de Fe, Zn e Se, nos últimos 100 anos (GARVIN; WELCH; FINLEY, 2006).

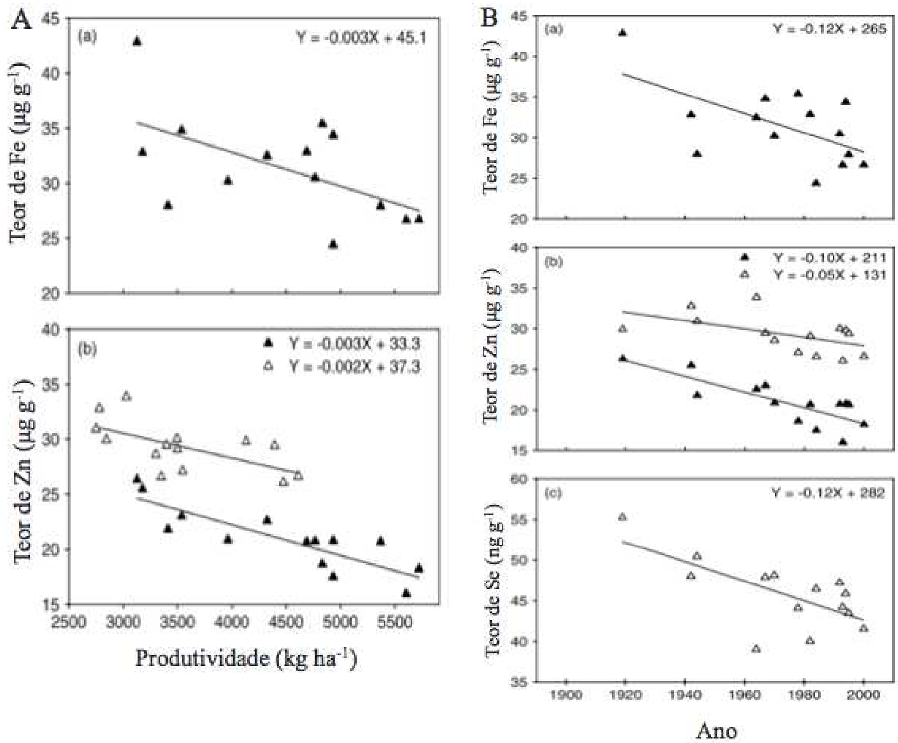

Figura 1 A) Teores de Fe e Zn em plantas de trigo em função da produtividade;
B) Teores de Fe, Zn e Se, em plantas de trigo, nos últimos 100 anos.
Adaptado de Garvin, Welch e Finley (2006).

A biofortificação é o processo pelo qual ocorre o aumento do teor de nutrientes, em produtos agrícolas a serem colhidos e/ou posterior a essa etapa, na industrialização, visando o enriquecimento do produto final com determinado elemento (GÓMEZ-GALERA et al., 2010; WELCH, 2005; WHITE; BROADLEY, 2005). Culturas biofortificadas podem conter características agronômicas e nutricionais melhores em relação às que não passaram por esse processo (WELCH; GRAHAM, 1999). A biofortificação faz sentido, pois é parte de um enfoque que considera um sistema alimentar integrado, visando reduzir a desnutrição e tem como alvo a população mais necessitada. É cientificamente viável e efetiva em termos de custos, 20 dólares por pessoa, além de complementar outras intervenções para o controle das deficiências de Fe, Zn, I, Se e vitamina A. Ainda, devem-se considerar os benefícios (viabilidade, vigor das mudas, estandes mais uniformes, melhor uso da água e mais resistência a doenças) e retornos econômicos aos produtores (BOUIS et al., 2011; BOUIS; WELCH, 2010; WELCH, 2005). É, portanto, o primeiro passo que possibilitará uma melhoria na nutrição e saúde de famílias carentes de maneira sustentável (HARVASTPLUS, 2004). Para aumentar os teores de nutrientes em plantas, é preciso compreender os processos fisiológicos e moleculares que fundamentam os estudos em Nutrição Mineral de Plantas (CAKMAK, 2008; GRUSAK, 2002; WHITE; BROADLEY, 2009).

A fortificação industrial bem como a suplementação por meio de medicamentos tem sido um dos melhores processos para o controle de deficiências de micronutrientes em crianças, em todo o mundo. No entanto, os altos custos e riscos de intoxicações causados por uma superdose são maiores nesse caso (ZANCUL, 2004).

O restrito acesso das populações aos alimentos ricos em micronutrientes, a presença em proporções inadequadas de inibidores e antinutrientes nas dietas, além da baixa biodisponibilidade dos minerais, são causas atribuídas às deficiências desses elementos na população (FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS - FAO, 2000; LONG; BA; SMITH, 2004). Estima-se que três bilhões de pessoas apresentam alguma deficiência de Fe, Zn, I, Se e vitamina A (GRAHAM; WELCH; BOUIS, 2001; WHO, 2002). De acordo com o *Copenhagen Consensus* 2008, deficiências de Zn e vitamina A são e devem ser tratadas como prioridades em países em desenvolvimento (www.copenhagenconsensus.com). Portanto, esses elementos estão como prioridades no combate à desnutrição humana (GRAHAM;

WELCH; BOUIS, 2001). Outros minerais, como cálcio (Ca), magnésio (Mg) e cobre (Cu) podem ser deficientes na dieta de algumas populações (WELCH; GRAHAM, 2004; WHITE; BROADLEY, 2005).

O Brasil ainda não possui resultados confiáveis sobre tal assunto, além disso, as diferenças econômica e social entre as pessoas que aqui vivem, dificulta um estudo mais abrangente que correlacione toda a população nacional. Assume-se que a deficiência de Fe é a mais generalizada, principalmente entre crianças e gestantes, além disso, as deficiências de Zn e Se devem ser semelhantes ao padrão internacional, ou seja encontram-se deficientes (MORAES, 2008).

2.2 Zinco (Zn) em solos, plantas e humanos: um histórico de deficiências

2.2.1 O Zn nos solos

O Zn é um metal de transição com número atômico 30, pertencente ao grupo II B, da tabela periódica. É o 23º elemento mais abundante na crosta terrestre e apresenta-se sob a forma Zn^{+2}, sendo adsorvido na superfície das partículas do solo com argilas, matéria orgânica e óxidos de Fe (MALAVOLTA, 2006). Os teores médios desse elemento nos solos encontram-se entre 60 e 89 mg kg^{-1} (BROADLEY et al., 2007; KABATA-PENDIAS, 2011). Contudo, os valores na solução do solo são baixos e estão diretamente relacionados com a textura do solo, pH, matéria orgânica, atividade microbiana e concentrações de fósforo e elementos catiônicos (ALLOWAY, 2009).

Os valores naturais de Zn, nos solos, variam de acordo com a rocha de origem e de fontes de deposições, tais como vulcões, queimadas naturais e deposição de poeira. Contudo, as atividades antropogênicas e o cultivo continuado desses solos têm aumentado de forma considerável os teores de Zn nos mesmos (ALLOWAY, 2008; BROADLEY et al., 2007). Além disso, é sabido que práticas agrícolas podem aumentar os teores de Zn nos solos (e.g. adubação, calagem, rotação de culturas, cultivo mínimo e plantio direto), embora outras, tais como calagem e adubação fosfatada possam agravar a deficiência (GRAHAM et al., 2007). Rassalta-se que outros fatores podem afetar a disponibilidade de Zn nos solos, como por exemplo: a faixa de disponibilidade de Zn encontram-se entre pH 5,0 e 6,0; solos arenosos apresentam menor poder tampão, portanto se receberem doses de corretivos e o pH for elevado a 6,0, pode-se desenvolver deficiência; pode ocorrer a imobilização de Zn pela matéria orgânica, colóides do solo e por microrganismos; baixas temperaturas e excesso de umidade podem agravar a deficiência, no entanto os sintomas desaparecem posteriormente (ABREU; LOPES; SANTOS, 2007; LOPES, 1999; LOPES; GUILHERME, 1994). Embora as melhorias no manejo dos solos tenham sido adotadas pelos agricultores, estima-se que 50% dos solos cultivados com grãos são deficientes em Zn (ALLOWAY, 2008, 2009).

Adicionalmente, solos de regiões tropicais possuem alta fixação de P, do que resulta a necessidade do uso de adubações fosfatadas corretivas, que podem induzir à deficiência de Zn (ALLOWAY, 2009; LOPES, 1999; RAIJ, 2004). Recentemente, tem-se atribuído grande importância à inibição não competitiva e interações entre P e Zn em solos, onde incrementos na adubação de P induzem ao sintoma de deficiência de Zn nas folhas. Contudo, há um efeito de diluição de Zn nas plantas, que ocorre devido à maior produção de matéria seca provocado pelo acréscimo de P (ALLOWAY, 2009; KABATA-PENDIAS, 2011; MALAVOLTA, 2006).

2.2.2 O Zn nas plantas: teores, mecanismos de absorção e atuação

A essencialidade de Zn para as plantas foi determinada em 1926 em estudo realizados com plantas de beterraba e girassol (SOMMER; LIPMAN, 1926).

O Zn é o segundo elemento mais encontrado nos organismos e o único metal a participar dos seis grupos enzimáticos (BROADLEY et al., 2007). Devido a essas características, está envolvido na síntese dos ácidos nucléicos, DNA e RNA, sendo ainda um cofator enzimático que atua nos mecanismos de transcrição que compõem enzimas essenciais para o crescimento e diferenciação celular (HÄNSCH; MENDEL, 2009; MAYER; PFEIFFER; BEYER, 2008). Sendo assim, o Zn é um elemento crucial para vários processos metabólicos na planta, tais como a fotossíntese, síntese proteica, manutenção da integridade da membrana, metabolismo de auxina e reprodução. Além disso, atua na redução a formação de radicais livres por atuar em inibidores como a NADPH oxidase, indutor da metalotioneína, integrante da Cu-Zn-SOD, entre outras (CAKMAK, 2000; HÄNSCH; MENDEL, 2009; MAYER; PFEIFFER; BEYER, 2008).

A absorção de Zn pelas plantas é feita na forma de Zn^{+2}, contudo ainda discute-se se a absorção é passiva ou ativa, embora trabalhos relatem que a absorção do elemento é feita metabolicamente (ALLOWAY, 2008; MALAVOLTA, 2006). A concentração desse nutriente nas plantas varia conforme a espécie, contudo os teores para a maioria das espécies variam de 20 a 120 mg kg^{-1} de matéria seca (MALAVOLTA, 2006). Os teores foliares considerados adequados para as cultivares nacionais de trigo variam entre 20 e 40 mg kg^{-1} (MALAVOLTA, 2006; MALAVOLTA; VITTI; OLIVEIRA, 1997). Scheeren et al. (2011) avaliaram 180 cultivares de trigo em três locais e verificaram que os teores médios desse elemento nos grãos foi de 32 mg kg^{-1}, sendo a faixa de variação entre 21 e 55 mg kg^{-1}.

Os sintomas típicos da deficiência são relatados como encurtamento de internódios e produção de folhas novas pequenas, cloróticas e lanceoladas. Os sintomas de toxidez são raros e, no geral, são reportados como sendo diminuição da área foliar, seguida de clorose (MALAVOLTA, 2006; MALAVOLTA; VITTI; OLIVEIRA, 1997). Assim como descrito anteriormente, o pH, adubações com P, baixos teores de Zn e elevadas concentrações de elementos catiônicos são fatores limitantes à absorção do Zn, promovendo a deficiência desse em plantas.

O efeito da deficiência de Zn, em solos e plantas, ocorre em todo o mundo. Contudo, estudos evidenciam a resposta de plantas quando adubadas com Zn (CAKMAK, 2008; GRAHAM; WELCH; BOUIS, 2001), que varia de acordo com a espécie e genótipo (ALLOWAY, 2008; CAKMAK, 2008). Segundo os autores, genótipos eficientes conseguem absorver Zn de solos com baixos teores, produzindo mais matéria seca e grãos. Por outro lado, plantas cultivadas em solos deficientes nesse elemento podem ter sua produtividade reduzida drasticamente (ALLOWAY, 2008).

Plantas de trigo *durum*, cultivadas em solos com baixos teores de Zn disponíveis, apresentaram menores concentrações que plantas cultivadas em locais onde a disponibilidade desse nutriente era maior (CAKMAK; PFEIFFER; MCCLAFFERTY, 2010). A aplicação de Zn foliar e/ou no solo, em trigo, aumentou a produtividade e elevou os teores desse nutriente nos grãos de 11 para 22 mg kg^{-1}; a combinação Zn foliar e aplicação no solo elevaram os teores para 27 mg kg^{-1} (CAKMAK et al., 2010). Hussain et al. (2012) verificaram que a aplicação de Zn em solos aumentou a produção de grãos, a concentração de Zn nos grãos e a bioacessibilidade em 29%, 95% e 74% respectivamente. Os autores observaram que aplicações foliares e em solos, em diferentes épocas - perfilhamento e início do florescimento -, podem aumentar os teores de Zn nos grãos, contudo, quando aplicado no solo esses teores foram inferiores a 9 mg kg^{-1} de Zn.

2.2.3 O Zn e seus efeitos na nutrição humana

A essencialidade do Zn em humanos foi determinada em 1955, ao se relacionarem problemas cutâneos à decorrente deficiência de Zn (MAFRA; COZZOLINO, 2004). Tal qual ocorre para plantas, o Zn é essencial para o organismo de animais, incluindo o homem, desempenhando funções metabólicas e fisiológicas para o crescimento e desenvolvimento normal dos organismos. O Zn atua como agente anti-inflamatório, no sistema oxidativo, prevenindo doenças como: câncer, distúrbios neurológicos e doenças autoimunes. Além disso, estudos mais aprofundados relacionados à deficiência de Zn, bem como sobre a forma de suplementação com esse elemento, são necessários (CESAR; WADA; BORGES, 2005; JOMOVA; VALKO, 2011; MAFRA; COZZOLINO, 2004).

Em países em desenvolvimento, a deficiência de Zn está listada como o quinto fator de risco para a saúde humana, logo após o tabagismo, e no mundo encontra-se na 11ª posição (WHO, 2002). Em geral, cerca de 1,4% das mortes no mundo são atribuídas à carência de Zn, sendo 1,4% nos homens e 1,5% nas mulheres (WHO, 2006). Contudo, as informações a respeito da deficiência de Zn, em âmbito mundial, ainda são escassas e não muito precisas (HESS et al., 2009).

No Brasil, os valores diários de ingestão para adultos correspondem a 11 e 8 mg dia^{-1}, respectivamente para homens e mulheres (MAFRA; COZZOLINO, 2004). De acordo com os dados da Pesquisa de orçamentos familiares 2008-209 (POF 2008-2009), há uma prevalência de inadequação de 24% para homens e 22% para mulheres. Vale ressaltar que esses valores foram determinados em função de hábitos alimentares da população nacional. No entanto, os valores relacionados a Zn foram obtidos a partir de dados norte-americanos e da tabela brasileira de composição de alimentos (INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA - IBGE, 2011).

Os três fatores responsáveis pela deficiência de Zn, em países de baixa renda, são a ingestão inadequada na dieta ou uma dieta composta, principalmente, de vegetais. Outros fatores podem agravar essa deficiência, principalmente em crianças onde há amamentação com baixa quantidade de nutrientes e sob o risco de doenças, que induzem a perdas excessivas ou prejudicam a utilização de Zn em estados fisiológicos, como o crescimento, a infância e a gravidez (HESS et al., 2009; MAFRA; COZZOLINO, 2004). É sabido que Zn e cádmio (Cd) possuem interações em solos, plantas e animais, no entanto, autores divergem a respeito se essas são sinérgicas ou antagônicas (KABATA-PENDIAS, 2011). Dados recentes mostram que pessoas que apresentam baixa renda, são fumantes e/ou encontram-se em contato com a fumaça desses cigarros são mais susceptíveis à intoxicação por Cd (TYRRELL et al., 2013). No entanto, com uma diversificação alimentar e incrementos de alimentos com maiores teores de Zn, pode ocorrer uma menor toxidez causada pelo Cd, em parte da população, devido ao antagonismo entre Zn e Cd.

2.3 Selênio (Se) em solos, plantas e humanos: uma potencial preocupação

2.3.1 O Se nos solos

O Se pertence ao grupo dos calcogênios (grupo 16 da tabela periódica) e está naturalmente presente sob 5 formas de oxidação (FERNÁNDEZ MARTÍNEZ; CHARLET, 2009; ZHANG et al., 2008). Os teores disponíveis desse elemento nos solos são baixos, na faixa de 0,05 e 1,5 mg kg^{-1}. Entretanto, áreas seleníferas podem apresentar concentrações acima de 1.200 mg kg^{-1} (KABATA-PENDIAS, 2011).

Assim como relatado para o Zn, os teores de Se no solo são controlados principalmente pelo material de origem. Contudo, outros fatores como pH, potencial redox, a forma química ou espécies de Se, a textura e mineralogia do solo, o teor de matéria orgânica e a presença de íons competitivos exercem influência nesses teores (KABATA-PENDIAS, 2011; QIN; ZHU; SU, 2012). Os aumentos nas concentrações de Se nos solos ocorrem devido a deposições atmosféricas, principalmente em áreas litorâneas e com altas atividades vulcânicas, volatilização e queima de vegetação selenoferrosas e atividades antropogênicas, tais como industrialização e aplicações via fertilizantes (KABATA-PENDIAS; PENDIAS, 2001). O principal programa de fertilização de Se, em solos, ocorreu entre os anos de 1984 a 1990 na Finlândia onde, em área de produção de grãos, foram aplicadas, via fertilizante, doses anuais de 8 g ha^{-1} e 3 g ha^{-1} de selenato de sódio para produção de grãos e pastagens, respectivamente (ARO; ALFTHAN; VARO, 1995).

Em condições naturais, as principais formas de Se são o selenito (Se^{+4}) e selenato (Se^{+6}). O primeiro é adsorvido na superfície das partículas do solo com maior afinidade que o segundo, especialmente em baixo pH e na presença de óxido de Fe e matéria orgânica. Dessa forma, o selenito é menos biodisponível que o selenato, sendo esse mais solúvel, móvel e facilmente disponibilizado para a absorção de plantas em solos neutros a alcalinos (KABATA-PENDIAS, 2011).

Estima-se que as formas de Se adsorvidas pelo solo ficam na faixa de 15 a 40% do seu teor total e que o Se orgânico varia de 4 a 22% do teor total (KABATA-PENDIAS; PENDIAS, 2001). A adsorção de Se em solos pode ser reduzida por fosfatos e sulfatos, especialmente em solos ricos em óxidos de Fe e Mn (KABATA-PENDIAS, 2011; KABATA-PENDIAS; PENDIAS, 2001).

Os teores de Se em solos brasileiros são baixos, o que pode evidenciar a carência desse elemento em algumas regiões do país (FERREIRA et al., 2002). Para determinar os teores de Se em solos de cerrado de Minas Gerais e Goiás, sob vegetação nativa (n=90) e duas profundidades (0-20cm e 80-100cm), Carvalho (2011) verificou que os teores desse elemento variaram entre <22 a 81 μg kg^{-1}. Vale ressaltar que esse bioma corresponde a 23% da área total do país e onde se concentram as principais áreas agrícolas do país. Em amostras de solos coletadas no estado de São Paulo (n=58), camada de 0-20cm, Gabos (2012) verificou que os teores de Se nesses solos variaram entre <0,08 a 1,61 mg kg^{-1}, sendo que a média ficou próxima a 0,19 mg kg^{-1} de Se.

2.3.2 O Se nas plantas: teores, mecanismos de absorção e atuação

Para plantas, o Se não tem sua essencialidade comprovada, no entanto, há extensos relatos a respeito do seu efeito benéfico. Encontram-se, por exemplo, estudos relatando que, em baixas concentrações, esse elemento pode aumentar a atividade antioxidande nas plantas, levando a uma maior produção e produtividade (DJANAGUIRAMAN et al., 2005; LI; MCGRATH; ZHAO, 2008; RAMOS et al., 2010).

O Se do solo determina a sua concentração em grãos e tecidos vegetais (LI et al., 2008). Apesar disso, a quantidade de Se nos solos e nas culturas depende de uma série de fatores como, por exemplo, geológicos, geográficos, pH do solo, potencial redox, matéria orgânica, competições iônicas - como ocorre com o sulfato - umidade, temperatura e textura do solo (CUBADDA et al., 2010). Aliás, tanto a absorção quanto o acúmulo desse pelas plantas não dependem apenas do teor no solo, mas também da forma com que o elemento se encontra (LI et al., 2008). O Se é absorvido pelas plantas tanto na forma de selenito como de selenato. A absorção do primeiro se dá de forma passiva e, provavelmente, os transportadores de fosfato estão envolvidos, enquanto o selenato é absorvido de forma ativa e utiliza os transportadores de sulfato nos processos (LI; MCGRATH; ZHAO, 2008; ZHU et al., 2009).

As culturas respondem diferentemente à adubações com selenato e selenito. Para a maioria das culturas, o selenato apresenta aspectos menos tóxico que o selenito (LI; MCGRATH; ZHAO, 2008; ZHU et al., 2009). Aplicações de Se em vasos com solo na forma de selenato de sódio, em doses maiores que 2,6 mg kg^{-1}, mostraram-se tóxicas para o trigo, pois reduziram o crescimento em 10%, quando comparadas com a dose controle de 2,0 mg kg^{-1} (LYONS; STANGOULIS; GRAHAM, 2005). A aplicação de um grama de Se por hectare, em solos do Reino Unido, aumentou a concentração de 16 para 26 ng kg^{-1} de Se em grãos de trigo (BROADLEY et al., 2010). Segundo os autores, a aplicação de 10 g de Se por hectare aumentaria em 10 vezes a concentração de Se nos grãos de trigo, considerando-se os níveis atuais nos solos da Grã-Bretanha. Aplicações foliares na Austrália - até 330 g ha^{-1} de Se - não foram tóxicas para o trigo (LYONS et al., 2005). No entanto, Ducsay e Ložek (2006) afirmam que a dose de 10 g ha^{-1} de Se é suficiente para se elevar a níveis satisfatórios os teores desse elemento, para a cultura do trigo.

Devido à sua importância e essencialidade para humanos, aplicações de Se, visando a biofortificação agronômica de plantas, têm sido amplamente difundidas na literatura, nos últimos anos (LYONS, 2010; LYONS et al., 2005; LYONS; STANGOULIS; GRAHAM, 2003).

2.3.3 O Se e seus efeitos na nutrição humana

Ao contrário do que acontece para as plantas, o Se é essencial para animais e humanos. Sua essencialidade foi comprovada em 1957, quando esse promoveu a defesa hepática de ratos deficientes em vitamina E (ZENG, 2009). O Se presente na dieta animal, incluindo a do homem, tem sua origem nos vegetais e grãos, seja pelo consumo "*in natura*" ou pelo consumo de carnes (BITTERLI; BAÑUELOS; SCHULIN, 2010).

Estima-se que 15% da população mundial seja deficiente em Se e a principal razão é a baixa ingestão desse nutriente (WHITE; BROADLEY, 2005). Portanto, é grande o interesse de pesquisadores em estudos envolvendo esse nutriente e seus efeitos para a saúde humana, atualmente. Sabe-se que o Se atua como cofator enzimático e no controle da eliminação de radicais peróxidos do organismo. Além disso, possui atividades anti-inflamatórias, químio-preventivas e antivirais (PAPPAS et al., 2008).

A baixa ingestão de Se está ligada ao desenvolvimento de doenças cardiomiopáticas (doença de Keshan-Beck), hipotireoidismo e redução da atividade do sistema imunológico. No geral, essas doenças são relatadas em áreas onde as concentrações desse elemento nos solos são baixas, como China, Tibete e Sibéria (ELLIS; SALT, 2003; ZENG, 2009). Ademais, a suplementação com Se diminuiu a incidência de câncer de colo, próstata e pulmão. Contudo, mais estudos são necessários para que se comprove uma correlação entre o Se e o câncer em humanos (WHANGER, 2002). Por outro lado, o Se tornou-se conhecido por seus efeitos tóxicos em animais e humanos (ELLIS; SALT, 2003; FORDYCE et al., 2010).

No Brasil, não existem levantamentos sobre a deficiência de Se na população, mas reporta-se que os teores ingeridos sejam baixos em relação ao padrão internacional (MORAES, 2008). No entanto, a POF 2008-2009 inferiu, para esse nutriente, os níveis de ingestão diários para adultos e os valores encontram-se entre 98 e 76 μg dia^{-1}, respectivamente para homens e mulheres. Assim como acontece para Zn, os teores de Se na POF 2008-2009 foram determinados a partir da ingestão média desse elemento em função dos hábitos alimentares e nos teores desse elemento presentes na base de dados norte-americana (IBGE, 2011). Para o Se, esses dados têm que ser analisados com certa ressalva, pois os teores desse elemento na alimentação entre os dois países, são muito diferentes. A ingestão diária recomendada de Se deve ser 55 μg dia^{-1}, contudo, concentrações inferiores a 25 μg dia^{-1} têm sido reportadas (ZENG, 2009). Portanto, para prevenção, indica-se a adição desse elemento em dietas, via suplementação em sais inorgânicos, principalmente selenato de sódio, ou formas orgânicas enriquecidas com Se (LYONS; STANGOULIS; GRAHAM, 2003; WANG et al., 2011). Além disso, vale ressaltar que a disponibilidade desse nutriente pode variar de acordo com a forma mineral e/ou iônica (WANG et al., 2011).

2.4 Interações Zn x Se em plantas, humanos e animais

Interações entre micronutrientes podem afetar a absorção e a biodisponibilidade, por uma série de fatores. Elementos quimicamente semelhantes podem concorrer por sítios de transporte de proteínas, mecanismos de absorção e por compostos quelatizantes, facilitando ou não a absorção (SANDSTRÖM, 2001). Embora não sejam quimicamente similares, Zn e Se são essenciais para o bom funcionamento do organismo animal e realizam funções semelhantes, a de antioxidantes (BETTGER, 1993).

Em humanos, a interações Zn x Se têm sido amplamente estudadas nas áreas biomédicas (BLESSING et al., 2004; DANSCHER; STOLTENBERG, 2005). Blessing et al. (2004) estudando os efeitos de compostos de Se na atividade de proteinas do DNA ("zinc fingers"), verificaram que essas proteínas são altamente reativas com compostos oxidantes de Se, o que poderia afetar a expressão gênica, reparação do DNA e, consequentemente, uma desestabilidade genômica. Estudos revelam ainda que a metationeína é oxidada pela glutationa peroxidase, com presença de formas orgânicas de Zn, sugerindo assim um controle da reação redox por esse metal (JACOB; MARET; VALLEE, 1999).

Para animais, os efeitos de Zn, Se e a sua combinação (Zn x Se) foram avaliados em ratos sob diabetes induzida e concluiu-se que a suplementação de dietas com Zn, Se e a combinação Zn x Se aumentou o peso, facilitou a absorção de glicose, preveniu o estresse oxidativo, reduziu a peroxidação lipídica e preservou as funções hepáticas nos ratos (UKPERORO et al., 2010). Por outro lado, House e Welch (1989) verificaram, em solução nutritiva, que em plantas de trigo submetidas a doses de Zn e Se, os teores do primeiro não afetaram o segundo e vice-versa. Os mesmos autores, ao estudarem a bioacessibilidade de Zn e Se em ratos, alimentados com o trigo, verificaram que Se possui efeito antagônico na absorção de Zn e o contrário também foi observado. A literatura relata ainda que o Se inibe a taxa de crescimento de tumores de câncer em ratos, contudo, a aplicação de $ZnCl_2$ inibe o efeito protetor do Se contra os tumores (NORDBERG; ANDERSEN, 1981; SCHRAUZER; WHITE; SCHNEIDER, 1976).

2.5 Ferro (Fe) em solos, plantas e humanos: os problemas além do solo

2.5.1 O Fe nos solos

O Fe é um metal de transição com número atômico 26, pertencente ao grupo VIII, da tabela periódica. É o segundo metal mais importante e um dos principais constituintes da crosta terrestre, sendo assim, o micronutriente com os maiores teores em solos, em torno de 5% (KABATA-PENDIAS, 2011; MALAVOLTA, 1980). No entanto, em solos brasileiros, esses teores podem ultrapassar a 10%. Dessa forma, quando a deficiência de Fe aparece é devida a uma diminuição da disponibilidade ou na absorção e não pela falta do elemento (MALAVOLTA, 1980).

Em solos, está presente nas formas de materiais primários (e.g. silicatos, óxidos e carbonatos), secundários e adsorvidos pela matéria orgânica ou argilas. As concentrações na solução do solo são baixas e as formas predominantes são

Fe^{+2}; $Fe(OH)^{+2}$; $Fe(OH)_2^{+}$; $Fe(OH)_4^{-}$ e Fe^{+3} (ABREU; LOPES; SANTOS, 2007). De forma geral, a disponibilidade de Fe é maior em solos com baixo pH, sendo que aumento de uma unidade de pH reduz a disponibilidade de Fe em 1000 vezes. Além disso, fatores como: baixos teores de Fe; presença de P, Cu, Mn e Zn; matéria orgânica e encharcamento podem reduzir a disponibilidade desse elemento (MALAVOLTA, 1980, 2006).

De certa forma, a coloração dos solos está relacionada com a presença de óxidos de Fe. Por exemplo, as colorações amarelas em regiões temperadas se devem a presença de goetita - óxidos mais hidratados, enquanto as cores vermelhas, mais comuns em regiões áridas, são devidas à hematita – óxido não hidratado (DECHEM; NACHTIGALL, 2006).

2.5.2 O Fe nas plantas: teores, mecanismos de absorção e atuação

A essencialidade de Fe para as plantas começou em 1844, quando Eusèbe Gris observou que a aplicação de Fe reduziu a clorose em folhas de parreirais. Apesar disso, a determinação desse critério foi feita em 1860 por Julius von Sachs, em experimentos conduzidos em solução nutritiva (ROMHELD; NIKOLIC, 2007).

A principal função do Fe é a de componente enzimático sendo que a maioria participa de processos de oxirredução. Além disso, o Fe participa dos dois principais grupos proteicos, as hemoproteínas onde participam de processos da respiração (e.g. catalase, peroxidase, superóxido dismutase e citocromos) e Fe-S proteínas, atuando em processos fotossintéticos (e.g. ferredoxina, nitrogenase e sulfato redutase) (DECHEM; NACHTIGALL, 2006; KOBAYASHI; NISHIZAWA, 2012; ROMHELD; NIKOLIC, 2007).

A absorção de Fe é feita sob as formas dos íons Fe^{+2}, Fe^{+3} e quelatizado. Contudo, a eficiência de absorção desse elemento está ligada à forma como ele encontra-se presente em solos e na eficiência de raízes em reduzir o Fe^{+3} para Fe^{+2} (MALAVOLTA, 2006). Aparentemente, esse processo é feito metabolicamente e há dois mecanismos diferentes envolvidos, um específico para dicotiledôneas (Estratégia I) e outro para gramíneas (Estratégia II) (BROADLEY et al., 2012; DECHEM; NACHTIGALL, 2006; KOBAYASHI; NISHIZAWA, 2012). Ainda, elevadas concentrações de metais catiônicos (e.g. Cu, Mn e Zn) podem reduzir a disponibilidade de Fe para as plantas devido à inibição competitiva (BROADLEY et al., 2012; MALAVOLTA, 2006).

As concentrações desse elemento na planta variam de acordo com a espécie, no entanto, para a maioria os teores encontram-se entre 10 a 1.500 mg kg^{-1} de matéria seca (DECHEM; NACHTIGALL, 2006; KABATA-PENDIAS, 2011). Por se tratar de um elemento pouco móvel na planta, os sintomas típicos de deficiência aparecem em folhas novas, as quais caracterizam-se por apresentarem uma rede verde fina das nervuras sobre um fundo amarelo-claro. O que é explicado pela baixa síntese de clorofila e, com a evolução dos sintomas, as folhas podem apresentar um branqueamento (MALAVOLTA, 2006; MARSCHNER, 1995).

Em decorrência dos elevados teores de Fe encontrados nos solos, principalmente os brasileiros, problemas relativos à toxidez são mais comuns que os de deficiências, sobretudo em solos sob inundação. Entretanto, em solos alcalinos, a presença de metais catiônicos e adubações com excesso de P são fatores limitantes à absorção de Fe, promovendo a deficiência desse em espécies vegetais (ABREU; LOPES; SANTOS, 2007; DECHEM; NACHTIGALL, 2006). Por esses motivos e os diferentes mecanismos de absorção de Fe (Estratégia I ou Estratégia II), recomendações de adubação, principalmente via foliar, são indicadas.

Trabalhos envolvendo adubação com Fe são escassos e, no geral, envolvem temas relativos à biofortificação ou à mobilidade desse nutriente nas plantas. Scheeren et al. (2011) avaliaram 180 cultivares de trigo em três locais e verificaram que os teores médios desse elemento nos grãos foi de 33 mg kg^{-1}, sendo a faixa de variação entre 22 e 56 mg kg^{-1}. Em experimentos de casa de vegetação, com trigo, foi verificado que 77% do Fe foi translocado da parte aérea para os grãos, na maturação (GARNETT; GRAHAM, 2005). Além disso, aplicações foliares de Fe indicam o incremento de produtividade nos teores e no transporte desse nutriente, na parte aérea e nos grãos (CAKMAK; PFEIFFER; MCCLAFFERTY, 2010; HABIB, 2009; ZHANG et al., 2010).

2.5.3 O Fe e seus efeitos na nutrição humana

Assim como descrito para Zn e Se, o Fe também é essencial para humanos. Está envolvido em atividades metabólicas e enzimáticas, crescimento celular, reações da cadeia respiratória, síntese de DNA e funções do sistema imunológico.

Estima-se que 60% da população mundial seja carente desse nutriente, o que a torna a principal preocupação e a mais estudada no que se refere à saúde humana (GURZAU; NEAGU; GURZAU, 2003; WHITE; BROADLEY, 2009; WHO, 2006). O sintoma mais comum dessa deficiência é a anemia, mas também afeta o crescimento e o desenvolvimento, o sistema imune e as funções neurais (ARREDONDO; NÚÑEZ, 2005; WHITTAKER, 1998). Dentre os principais grupos de risco encontram-se as mulheres grávidas, recém-nascidos, crianças e adolescentes (WHO, 2002).

Assim como descrito anteriormente para Zn e Se, a deficiência de Fe também ocorre devido aos baixos teores bioacessíveis presentes nos alimentos que compõem a dieta básica (LYNCH, 2011). Ainda, a forma química presente, as células mucosas e da parede do intestino, bem como a quantidade ingerida, influenciam nos teores adquiridos do elemento (GURZAU; NEAGU; GURZAU, 2003). No entanto, políticas públicas visando o enriquecimento de farinhas e féculas de cereais com esse nutriente vêm sendo implementadas em diversas partes do mundo, principalmente em países em desenvolvimento (MANNAR; GALLEGO, 2002), fato que não ocorre para Zn e Se.

No Brasil, de acordo com dados da POF 2008-2009, os níveis de ingestão de Fe para adultos encontram-se entre 13,5 e 10,1 mg dia^{-1}, respectivamente para homens e mulheres. Mais uma vez, destaca-se que a composição de Fe nos alimentos foram determinadas de acordo com hábitos alimentares e utilizando-se as tabelas norte-americana e nacional de composição de alimentos (IBGE, 2011). A ingestão diária recomendada para homens é de 8 mg kg^{-1} e para mulheres 18 mg dia^{-1}, no entanto, se a mesma é gestante ou lactante os valores são, respectivamente, 27 e 9 mg dia^{-1} (BORTOLINI; FISBERG, 2010). Segundo os autores, o cálculo mulheres adultas levaram em consideração as perdas referentes ao período fértil. Para gestantes, consideraram-se as perdas basais de Fe, tais como os depositados no feto e tecidos relacionados e para a expansão da massa de hemoglobina. Para as lactantes, os cálculos basearam-se no fato de que, após seis meses de aleitamento, a menstruação retorna ao ciclo normal.

Para prevenção, indica-se a adição desse elemento em dietas, bem como ingestão de carnes e vitaminas A e C que são facilitadores da absorção de Fe (BORTOLINI; FISBERG, 2010). Ressalta-se ainda, que a presença de antinutrientes (fosfatos, fitatos e polifenóis), a forma química e a quantidade absorvida afetam a disponibilidade de Fe (BORTOLINI; FISBERG, 2010; GURZAU; NEAGU; GURZAU, 2003).

2.6 A cultura do trigo

Dentre os cerais, o trigo (*Triticum aestivum*) é a cultura mais importante (PENG; SUN; NEVO, 2011), não só por ser a primeira espécie domesticada pelo homem, mas também por compor 30% dos carboidratos presentes na dieta humana (SHEWRY, 2009; WELCH et al., 2005).

O primeiro cultivo tritícola ocorreu na região do Mediterrâneo há, aproximadamente, 10 mil anos atrás, quando o homem deixou de viver exclusivamente da caça e coleta de alimentos. De acordo com os autores, os primeiros cultivos foram compostos por plantas diploides (*einkorn*) e tetraploides (*emmer*), selecionadas a partir de populações selvagens onde se procuraram as melhores características produtivas. A partir desse momento, o cultivo de trigo se intensificou e o melhoramento genético propiciou grande evolução nas cultivares e produtividade da cultura. Atualmente, as linhagens híbridas hexaploides correspondem a 95% do trigo cultivado no mundo (SHEWRY, 2009; TOUSSAINT-SAMAT, 2009).

O trigo é o segundo cereal mais produzido no mundo e, em condições ideais, lavouras de clima temperado, produzem de 8 a 10 t ha^{-1} (BRASIL, 2013; BRUM; HECK, 2005; SHEWRY, 2009). Por outro lado, as lavouras brasileiras possuem produtividades médias entre 3 e 4 t ha^{-1} (BRUM; HECK, 2005). No Brasil, o trigo é cultivado durante o inverno e primavera, ocupando três regiões do país: Sul (RS, SC e PR), Sudeste (SP e MG) e Centro Oeste (MS, GO e DF). O cultivo desse cereal em áreas do cerrado vem crescendo e só é possível, principalmente pelo uso da irrigação (CAIERÃO, 2013; DALMAGO et al., 2013) e melhoramento genético feito na Embrapa Cerrados.

A estimativa da produção mundial para o ano de 2013 é de 701 milhões de toneladas, sendo os principais produtores China, Índia e Rússia (FAO, 2013). Segundo a FAO (2013), o consumo mundial desse cereal apresenta crescimento de 2,1% ao ano, enquanto a produção 1,5%. A produção nacional, para o ano de 2013, está estimada em 6 milhões de toneladas, gerando um déficit entre 4 e 5 milhões de toneladas, em relação ao consumo (COMPANHIA NACIONAL DE ABASTECIMENTO - CONAB, 2013). Em 2009, o Ministério da Agricultura criou políticas de incentivo para produção de trigo visando à autossuficiência do país nesse cereal e, um aumento gradativo na produção foi verificado no ano seguinte. No entanto , o próprio ministério estima aumento anual de 1,3% no consumo nacional (BRASIL, 2011). No ano de 2011, apesar de não figurar entre os maiores produtores de trigo, o Brasil ocupou o 18º lugar no comércio mundial desse cereal (FAO, 2013).

3 CONSIDERAÇÕES GERAIS

Temas referentes à segurança alimentar estão tornando-se frequentes na literatura científica mundial, destacando-se, dentre eles, a biofortificação, a qualidade alimentar e a contaminação de alimentos por metais e compostos orgânicos. Portanto, faz-se necessário que o Brasil, como grande produtor de alimentos do mundo, esteja atento às questões futuras, principalmente no tocante à qualidade dos produtos produzidos.

Nas duas etapas desse trabalho, foram abordados apenas a biofortificação com Fe, Zn e Se na cultura do trigo, cultura da qual o país importa quase 50% do total consumido. Os impactos iniciais desse trabalho serão, *a priori*, internos; 1) na qualidade de vida dos brasileiros, visto que as cultivares utilizadas são nacionais e, 2) na balança comercial agrícola nacional, uma vez que toda a nossa produção é aqui consumida. Entretanto, vale ressaltar que, para várias culturas há a tendência que se pague por qualidade do produto colhido. Assim, num futuro bem próximo, acredita-se que, para grãos destinados ao consumo humano, minimamente processados ou "in natura" - casos de trigo, milho, soja, arroz, feijão, etc. - as empresas comecem a pagar por qualidade e os nutrientes presentes podem ser o diferencial a favor do agricultor brasileiro.

Estudos envolvendo biofortificação com elementos como K, Ca, Mg, B, Cu, Fe, Mn, Zn, Cr, Se e I são necessários, não apenas para contribuir com a qualidade do produto final, mas também pensando nos efeitos benéficos à saúde humana. Ressalta-se que, além de enriquecer os alimentos com determinados elementos, faz-se necessário reduzir a quantidade de antinutrientes (e.g. fitatos, polifenóis, fibras e metais tóxicos), fator tão importante quanto o enriquecimento com alguns nutrientes.

Para serem adotadas políticas públicas de interesse nacional, como é o caso da biofortificação, são necessárias outras medidas, tais como: mapeamento da deficiência de nutrientes na população brasileira e distribuição geográfica dessas, além de incentivos à agricultura regional para que se desenvolvam produtos adaptados à determinada região. Entretanto, como mencionado anteriormente, há evidências de que a população brasileira seja carente nos elementos abordados nesse trabalho, o que justificaria qualquer esforço envolvendo o enriquecimento de culturas com alguns elementos.

Finalmente, o Instituto Brasileiro de Geografia e Estatística (IBGE) lançou a Pesquisa Nacional de Saúde 2013 (PNS) para saber como está a saúde e o estilo de vida do brasileiro. Serão escolhidos, aleatoriamente, 25% dos entrevistados, maiores de 18 anos, para coleta de medidas antropométricas e de pressão arterial. Além disso, serão coletados amostras de sangue e urina onde se analisarão diabetes, colesterol, anemias, doenças renais e sódio. Uma questão pode ser levantada: Não seria um bom momento para se analisar os teores de nutrientes nessas amostras que estão sendo coletadas? Quando será realizado outro sistema de coleta de abrangência nacional para que se tenham essas informações? Mais uma vez, pesquisa e política pública encontram-se distantes, o que gerará um enorme atraso para solução de problemas nutricionais dos brasileiros.

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SEGUNDA PARTE

Artigo 1

Evaluation of germplasm effect on Fe, Zn and Se content in wheat seedlings

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Evaluation of germplasm effect on Fe, Zn and Se content in wheat seedlings

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Abstract

Micronutrients are essential for human health and crucial for plant survival. The capacity of food crops in acquiring mineral nutrients affects plant growth and potentially the yield and nutrient content in edible tissues/organs. In this study, we selected 20 wheat (*Triticum aestivum* L.) accessions and evaluated genotypic variations of the young seedlings in response to iron (Fe), zinc (Zn), and selenium (Se) treatments. Wheat accessions exhibited different growth responses to these minerals and possessed various abilities to accumulate them. Wheat seedlings in general were less tolerable to excess of Fe and benefits from increased levels of Zn supply. They were sensitive to selenite and profited from selenate treatment at low dosages. Limited mineral interactions were observed between Fe or Zn with other nutrients. In contrast, selenate supply enhanced Fe, Zn, sulfur (S), molybdenum (Mo), magnesium (Mg), calcium (Ca) and manganese (Mn) content in wheat seedlings, supporting its beneficial role in promoting plant growth; Selenite supplement reduced Zn, S, Mo, Mg, Ca and Mn levels in the plants, consisting with its detrimental role in inhibiting seedling growth. Based on nutrient content accumulated, plant growth, and mineral interaction, a number of accessions such as EMB 38 and BRS 264 appeared to be excellent lines for breeding wheat cultivars with better plant health and potential to accumulate essential micronutrients in edible grains.

Keywords

Wheat (*Triticum aestivum L.*), iron, zinc, selenium, mineral interaction.

1. Introduction

Micronutrient malnutrition is a well-known human health problem, which affects more than 3 billion people in the world [1]. Deficiencies in iron (Fe), zinc (Zn), and selenium (Se) are common and estimated to affect approximately 60%, 30%, and 15%, respectively, of the world population [2]. Biofortification of crops with enhanced mineral content provides a complementary strategy in conjunction with dietary diversification, mineral supplementation, and food fortification to address the worldwide micronutrient deficient problem [2,3]. Micronutrients are important not only for human health, but also for plant nutrition. Fe and Zn are essential micronutrients for the survival and proliferation of all plants. Although the essentiality of Se to plants has not been established yet, Se is considered a beneficial element in promoting plant growth in some plant species [4,5].

Iron is indispensable for a variety of cellular functions in plants, participating in photosynthesis, respiration, chlorophyll biosynthesis, DNA synthesis, and hormone synthesis [6,7]. Fe is the second most abundant metal in soils and has extremely low solubility, especially in aerated alkaline soils [8]. Plants develop two distinct strategies for iron acquisition [7]. Graminaceous plants, such as wheat, use Strategy II to acquire Fe by secreting Fe-chelator compounds, mugineic acid family phytosiderophores. While low Fe availability causes leaf interveinal chlorosis, excess level of Fe is toxic to plants, believing to be due to the generation of oxidative stress [6,9]. Increase of Fe content in plants by fertilization is not very effective due to Fe insolubility in soil and low mobility of Fe in phloem [10].

Zinc serves as a cofactor in over 300 enzymes and is a structural component in many proteins, including a large number of transcription factors

[6,11,12]. It is estimated that approximately 50% of the cereal-grown area in the world have Zn-deficient soils. Zn level is dependent on available Zn pools and it status in plants directly correlates with plant growth, crop yield and nutritional quality [13]. Zn toxicity in crop plants are rare and Zn fertilization has been shown to be an effective approach to enhance Zn levels in leaves and in grains [10,13].

Selenium is uptake by plants from soils mainly as selenite (SeO_3^{-2}) and selenate (SeO_4^{-2}). Selenate is transported by an active process that is mediated by sulfate transporters. Selenite uptake appears to occur passively and phosphate transporters are believed to be involved in this process [5,14]. Most crops are sensitive to Se and respond differently to SeO_4^{-2} and SeO_3^{-2} fertilizations. While Se at high dosages inhibits plant growth, a number of studies have demonstrated that Se at low dosages enhances plant growth [15,16]. Selenium shares similar chemical properties with sulfur (S) and uses S uptake and assimilation pathways [5]. Thus, it is not surprised to find that high levels of Se fertilization suppress S accumulation [17]. However, studies have shown a synergistic instead of antagonistic relationship between Se and S metabolism at low dosages of Se supplement [18].

Wheat is the major staple crop that provides approximately 30% of food source for a large number of people worldwide. Improving the nutritional quality of wheat grain is highly important to help combat the global micronutrient deficiency problem. Toward this goal and to gain a better understand mineral nutrition in wheat plant, we evaluated the genotypic variation in response to the supplement of Fe, Zn and Se, the three trace elements whose deficiencies cause a major health problem worldwide. It is known that plants with high nutrient value in the leaf tissues can improve their growth, and consequently with a potential to increase yield as well as mineral content in edible grains [2,3]. We examined 20 wheat accessions from the wheat breeding program at Embrapa

Cerrados CPAC, Planaltina, Brazil. The effects of Fe, Zn, and Se supply on wheat seedling growth and nutrient content were investigated in order to provide the information for selection of wheat accessions with substantial capacity to acquire minerals and for crop biofortification with these micronutrients. Interactions among nutrients are known to impact plant nutrient status, plant health and yield [19,20]. The effects of Fe, Zn, and Se treatments on other nutrient accumulation in these wheat germplasm were also examined to provide a general view of mineral interactions in wheat plants.

2.1 Wheat germplasm, seedling growth, and mineral treatment

A population of bread wheat (*Triticum aestivum* L.) was selected from Embrapa Cerrados (CPAC) breeding program (Planaltina, GO, Brazil) and used in this study. This population included 20 accessions of 15 varieties and 5 cultivars. These lines were selected because they have high grain production in Central Brazil and contain good quality to bread industry. Their origin and pedigree are listed in Table 1. Seeds of each wheat variety or cultivar were surface-sterilized in 0.5% NaOCl for 15 minutes, rinsed in distilled milli-Q water, and germinated in a chamber at $20^{o}C$ for 3 days on moistened filter paper. The uniform 3-day-old young seedlings with roots of approximately 3 cm long were transplanted into 2.2 L pots containing modified Johnson's nutrient solution for wheat [21], which consisted of 1 mM KNO_3, 1 mM $Ca(NO_3)_2$, 0.05 mM $NH_4H_2PO_4$, 0.25 mM $MgSO_4$, 0.1 mM NH_4NO_3, 12.5 µM H_3BO_3, 0.1 µM Na_2MoO_4, 0.1 µM $NiSO_4$, 0.4 µM $MnSO_4$, 1.6 µM $CuSO_4$, 96 µM $Fe(NO_3)_3$, 10 µM $ZnSO_4$, 128 µM H_3HEDTA, and 2 mM MES buffer (pH 6.0). $ZnSO_4$ and $Fe(NO_3)_3$ were equilibrated with H_3HEDTA (N-2-hydroxyethylethhylenediamine-N,N',N'-triacetic acid) before adding to the nutrient solution. H_3HEDTA was used as micronutrient buffer.

Seedlings were grown with aeration in a greenhouse at 20^{o}C (±2^{o}C) under 16-h day length. Four days after transplant, these plants were either grown in the nutrient solution (control) or exposed to the nutrient solution with addition of 54 µM $Fe(NO_3)_3$ (totaling 150 µM Fe), 40 µM $ZnSO_4$ (50 µM Zn), 10 µM Na_2SeO_4 or 10 µM Na_2SeO_3. The additional Fe and Zn concentrations were used to achieve a final 50% free Fe activity (in consideration of potential Fe toxicity) and maximal free Zn activity in the culture solutions based on the calculation of using the GEOCHEM-EZ program [22]. A concentration at 10 µM of selenate and selenite was commonly used in nutrient solutions for non Se accumulator [14,15]. The solutions were changed twice each week. Following 10 days of treatments, a total of 400 plants (20 lines x 5 treatments x 4 repeats) were harvested individually and washed in distilled water. Shoots and roots were separated and dried at 65°C for 72 h.

2.2 Mineral analysis

Total mineral contents in the samples were determined using an inductively coupled plasma-emission spectrometer (model ICAP 61E trace analyzer, Thermo Electron, San Jose, CA). Individual dried shoot sample (200 mg) was weighed and pre-digested with 4 mL of concentrated $HNO_3/HClO_4$ (60/40% v/v) at room temperature overnight. The samples were then heated to 120ºC for 2 hours, followed by at 195ºC for 30 min. The samples were cooled to room temperature and diluted with milli-Q water to 20 mL. Blank and an internal standard Yttrium was used to substantiate the accuracy of the analytical results obtained. The experimental samples were prepared and analyzed following a rigid quality assurance/quality control (QA/QC) to ensure accurate and reliable analytical data.

2.4. Statistical analysis

All quantitative data on plant growth were mineral nutrient contents were subjected to statistical analysis by one way analysis of variance (ANOVA). The data were submitted to variance analysis by the Scott-Knott test at a level of 5% of probability, using the statistical analysis software SISVAR [23].

3. Results

3.1. Wheat accessions possess different growth responses to mineral treatments

Fe, Zn and Se are not only essential for human nutrition, but also exert profound effects on plant growth and development [2,3,10]. To examine the growth responses of these 20 selected wheat accessions to the application of these minerals, the shoot and roots of each wheat line were separately harvested and dried. Their biomasses were measured.

As shown in Figure 1A, these wheat lines exhibited up to 2-fold difference in their shoot biomass when they grew in the control nutrient solution. They responded differently to the additional supply of Fe and Zn as well as to supplement of selenate (SeO_4^{-2}) and selenite (SeO_3^{-2}) in the nutrient solution ($p \leq 0.05$). When the wheat lines were exposed to additional Fe, a general inhibition of shoot growth was observed in most of the lines. Four lines (EMB 30, EMB 34, Brilhante, and Supera) appeared to tolerate additional Fe supply with no inhibition on their shoot growth, and two lines (i.e. EMB 38 and BRS 264) were benefited by additional level of Fe fertilization with enhanced growth (Fig. 1A). Zn fertilization has been shown to enhance plant growth [13]. When the wheat lines were exposed to additional Zn, an enhancement of shoot growth was observed in half of these lines and an up to 2-fold increase in biomass was noticed in line EMB 19 and EMB 30 (Fig. 1A). Slight suppression of plant growth was observed in four lines (EMB 9, EMB 10, EMB 15, EMB 26). Plants respond differently to different form of Se and selenate is generally less toxic than selenite to plant growth [14,18]. When these wheat lines were grown in the nutrient solution containing 10 µM Na_2SeO_4, the plant growth was unaffected in some lines, and enhanced (EMB 7, EMB 14, EMB 19, EMB 30, EMB 38 and BRS 264) or suppressed (EMB 15, EMB 20, EMB 26, EMB 33, EMB 34, BRS 207, BRS 254, Supera) in the other lines (Fig. 1A). However, when the wheat varieties and cultivars were exposed to 10 µM $Na_2SeO_{3,}$ a general inhibition of plant growth was observed in nearly all lines except line EMB 5 and line EMB 30. An average of 36% suppression of growth was noticed. These results indicate that wheat lines exhibited genetic variation for shoot growth in response to mineral treatments. Each line responded differently to Fe, Zn and Se treatments, indicating a different capacity in uptake, assimilation and use of these nutrients within the plant.

Root dry matter of these wheat lines in response to Fe, Zn and Se treatments were also investigated. A similar response pattern as shoot growth was observed for root growth of these wheat lines when they were exposed to various mineral treatments ($p \leq 0.05$, Fig. 1B). Fe supplement at the dosage applied generally inhibited root growth, while additional Zn supply stimulated root growth. SeO_4^{-2} treatment had minimum effects on root growth in some lines and increased the root biomass in half of the other lines. A general reduction of root growth was observed in SeO_3^{-2} treated plants, consisting with that selenite is more toxic to plant growth than selenate.

3.2. Wheat accessions exhibit a range of capacity in the accumulation of minerals

To examine the responses of wheat cultivars and varieties to additional Fe and Zn as well as to different form of Se treatment, the total mineral content in the wheat seedlings were determined by ICP. The wheat lines accumulated different levels of Fe when they grew in the nutrient solution (Fig. 2A). An up to 3-fold variation was observed between wheat lines accumulating high and low level of Fe. The supplement of additional Fe caused an increased level of Fe content in half of these lines and an over 50% increase of Fe content was found in line EMB 10 and line EMB 11. The results showed genetic variation among these wheat lines in term of Fe concentration and capacity to accumulate Fe.

Zinc concentration also exhibited an over 2-fold variation among these wheat lines (Fig. 2B). The supplement of additional Zn dramatically increased Zn content in all lines. An up to 3-fold enhancement of Zn level was observed, indicating high capacities of these wheat lines to take up and accumulate Zn. The total Zn levels showed over 2-fold differences between accessions containing high and low levels of Zn. Wheat accessions derived from parental crossing with BRS 264 showed high capacity to accumulate Zn.

Selenium was undetected in seedlings of the wheat lines that grew in the control nutrient solution. When the seedlings of these wheat lines were exposed to SeO_4^{-2} and SeO_3^{-2}, the total Se levels showed 4- and 2-fold difference, respectively, between lines containing high and low level of Se (Fig. 2C). The SeO_4^{-2}-treated wheat seedlings contained a range of 3- to 7-fold more total Se than the SeO_3^{-2}-treated ones, consistent with previous reports that plants accumulate more Se when treated with SeO_4^{-2} than SeO_3^{-2} [14,18].

3.3. Interaction among mineral elements

To investigate whether supplement of one element affected the accumulation of macronutrients (P, K, Ca, Mg, and S) and micronutrients (B, Cu, Fe, Mn, Mo, and Zn), we measured these mineral content in response to Fe, Zn, SeO_4^{-2} and SeO_3^{-2} treatments and examined their interactions. A general overview of these mineral element interactions in these wheat lines is summarized in Table 2. P and K along with N are the primary macronutrients and high demand by plants. In general, P level did not change much in response to Fe, Zn and Se treatments in most of the wheat lines (Fig. 3A). The same is true for K level (Fig. 3B). Ca level was increased in half of these wheat lines following treatments with Fe and Zn, and in over half of them with SeO_4^{-2} treatment. Ca content was dramatically suppressed by SeO_3^{-2} in all accessions with an average of 33% reduction (Fig. 3C). Mg content was enhanced by Fe and SeO_4^{-2} in about half or over half of these lines, respectively, and inhibited by SeO_3^{-2} treatment in half of the lines (Fig. 3D). S level was not affected by additional Fe and Zn supply in these wheat lines (Fig. 3E). Se is known to effect S uptake and assimilation [5]. S concentration was dramatically enhanced with an average of 200% increase by SeO_4^{-2} at the dose applied for all wheat lines and inhibited by SeO_3^{-2} in majority of these wheat lines (Fig. 3E).

Examination of micronutrient content in these wheat lines revealed that in general B level did not change much in response to Fe, Zn and Se treatments (Fig. 3F). Cu level was also not affected by Fe, Zn and Se treatments in almost all the lines (Fig. 3G). While Fe content in most wheat lines was increased by additional supply of Fe to the nutrient solution, Fe level was stimulated by SeO_4^{-2} supplement in over half of the wheat lines (Fig. 3H). Fe content was suppressed by Zn treatment and was unaffected by SeO_3^{-2} treatment in majority of the lines (Fig. 3H). Mn level was enhanced in about half of wheat lines exposed to Zn and SeO_4^{-2} and dramatically reduced when plants were treated with SeO_3^{-2} with an average of 43% reduction (Fig. 3I). Mo concentration was generally not affected by further addition of Fe and Zn, but enhanced with an average of 71% increase by SeO_4^{-2} in all wheat lines and suppressed by SeO_3^{-2} in about half of the lines (Fig. 3J). Zn content was dramatically enhanced with additional Zn supply in all wheat lines (Fig. 3K). While Zn level was not generally affected by Fe application, SeO_4^{-2} caused a slight increase of Zn in half of the wheat lines, and suppressed by SeO_3^{-2} in majority of the lines (Fig. 3K).

4. Discussion

The new focus of plant breeding programs has been the development of genotypes with high nutrient content [10,24]. Genetic factors along with physiological factors determine crop nutrient uptake and subsequently the potential productivity and quality of food crops; and these factors vary among plant species, cultivars, tissues and organs [2,24]. Fe and Zn are essential micronutrients for plant growth and development. Fe and Zn deficiencies are widespread among plants grown in calcareous soils. Their deficiencies lead to serious loss of crop production and nutritional quality. While Se is considered to be non-essential for plants, Se at low dosages promotes plant growth. Exploration of germplasm variation and understanding of physiological basis in response to Fe, Zn and Se treatments are critical for breeding crop varieties with better capacities to utilize these minerals not only for better plant health and growth, but also for a potential of better crop nutritional quality [13,25,26].

The wheat accessions examined exhibited 2-fold difference in their capacity in utilization of nutrients for shoot and root growth. An overall correlated shoot and root growth was observed with these accessions. While only a few lines showed tolerance to additional levels of Fe, a general inhibition of shoot and root growth by excess Fe was observed. These results are consistent with other reports showing that wheat varieties have different capacity in utilization of Fe and high dosages of Fe suppress plant growth [25,27,28]. When these wheat lines were exposed to additional Zn supply, an enhanced shoot and root growth was observed in half of wheat accessions. Zn fertilization has been shown to enhance plant growth [13]. Wheat plants cultivated with six Zn doses (0, 0.025, 0.05, 0.1, 0.2 and 0.4 mg Zn kg^{-1} soil) increase shoots and roots dry matter [29], while wheat seedlings grown under Zn-deficient conditions reduce the shoot and roots biomass [30]. Up to 2-fold wheat genotypic variation in utilization of Zn was observed here, which is agreeable with previous studies [30]. Wheat accessions responded differently to different forms of Se. While the growth of these wheat lines was either stimulated/suppressed or unaffected when exposed to 10 μM SeO_4^{-2}, a general suppression of growth was found following treatment with the same concentration of SeO_3^{-2}. These results are consistent with previous studies showing that SeO_4^{-2} is less toxic than SeO_3^{-2} to plant growth [14,15]. Genetic variations among crop germplasm in response to SeO_4^{-2} treatment are also reported in previous studies. Filek et al. [31] showed that SeO_4^{-2} treatment causes a reduced growth in Polish wheat and an increased biomass in Finnish wheat. Although wheat lines exhibited genetic variation for shoot growth in response to Fe, Zn and Se treatments, each line responded differently to these mineral treatments, showing a different capacity in uptake, assimilation and use of these nutrients.

The wheat accessions exhibited 2- to 4-fold variation in total Fe, Zn, and Se content. The additional Fe and Zn supply increased Fe and Zn levels in half and all wheat lines, respectively, indicating a range of capacity of wheat lines in accumulating these mineral elements. Enhanced Fe levels following Fe treatment in some plants were also described previously [25,27,28]. Similarly, increased Zn concentration following Zn supply were observed in wheat [32]. SeO_4^{-2} supply was more effective than SeO_3^{-2} in inducing Se content in wheat seedlings. Plants treated with SeO_4^{-2} contained over 4-fold more total Se than those treated with same concentration of SeO_3^{-2}. These results are consistent with previous reports that plants accumulate more Se when treated with selenate than selenite [14,18].

Interactions among mineral elements affect plant nutrient status and consequently plant health and yield [19,20]. Despite of numerous studies, nutrient interactions are complex and still not well understood. Here we showed that the primary macronutrients P and K levels were generally not affected by additional Fe and Zn supplement and Se treatment in these wheat lines, although Zn has been shown to reduce P transfer from roots to shoots but enhance the transfer from shoots to grain in other studies [33]. In contrast, interactions between other macronutrients with Fe, Zn and Se were observed. Ca and Mg interacted positively with Fe, Zn and SeO_4^{-2} in half of these wheat lines. Ca content was dramatically suppressed by SeO_3^{-2} in nearly all lines, showing strong antagonistic interaction. No general interaction between S and Fe or Zn was noticed. S status in plants is known to be affected by Se supplementation [17,18,34]. The S levels were dramatically enhanced for all wheat lines at the SeO_4^{-2} dosage used, demonstrating a synergic interaction between SeO_4^{-2} and S. Similar results were observed in lettuce and *Arabidopsis* when Se was supplied at low dosages [18,35]. On the other hand, SeO_3^{-2} suppressed S accumulation in most of the wheat lines with antagonistic relationship as demonstrated in other studies [14,18,36].

Micronutrients in plants have gained a lot of attention recently due to their essential roles in plant growth and their contribution to human health [2,3,37]. Micronutrients B and Cu exhibited no interaction with Fe, Zn and Se in majority of these wheat lines. In contrast, Fe level in most of these wheat lines was reduced by additional Zn supplement with an antagonistic effect between Fe and Zn. The result is consistent with those studies showing interaction between Fe and Zn [20]. Fe content in these wheat seedlings was generally enhanced by SeO_4^{-2} treatment, showing a synergistic effect of selenate treatment on Fe accumulation. A slight enhancement in Fe content by SeO_4^{-2} was also reported in wheat [31]. Mn and Mo contents were also enhanced by SeO_4^{-2} treatment but suppressed by SeO_3^{-2} in half of these wheat lines. Interaction between Se and Mo is also reported in previous studies [34]. Noticeably, strong antagonistic interaction between Mn and SeO_3^{-2} and synergistic interaction between Mo and SeO_4^{-2} were observed in all these wheat lines. Interestingly, while interaction was observed between Fe and Zn with additional Zn supplement, Zn levels in wheat seedlings appeared not affected by additional Fe treatment in all these wheat lines. Previous studies in wheat plants show that Fe application decreased Zn uptake [19,20]. The discrepancy might be due to the nutrient status of wheat plants growing in nutrient solution vs. in soil. A slight increase and a general suppression of Zn level by SeO_4^{-2} and SeO_3^{-2}, respectively, were observed. Zn concentration is also found to be enhanced in wheat when selenate is applied [31]. It appears that supplement of SeO_4^{-2} at proper dosages caused synergic effects on the accumulation of several minerals, whereas supply of SeO_3^{-2} reduced the content of these elements in majority of the wheat lines. The synergic or antagonistic effects of different Se forms may contribute to the increased or decreased wheat seedling biomasses in these SeO_4^{-2} or SeO_3^{-2} treated plants.

5. Conclusions

Plant mineral status affects plant growth and health, potentially the yield and nutritional quality in edible tissue. By examination of these selected wheat accessions, we found that the wheat accessions showed genotypic variations in response to Fe, Zn and Se treatments. Thus, the study will be helpful in selecting and developing cultivars with better plant health and potentially high ability for consequent accumulation of essential micronutrients in wheat grains. Moreover, we found that wheat seedlings in general were less tolerable to high levels of Fe exposure, but benefits from increased levels of Zn supply. While the wheat seedlings showed suppressed growth when exposed to SeO_3^{-2}, they exhibited positive response to SeO_4^{-2} treatment, likely owing to the synergistic effects of SeO_4^{-2} in stimulating other mineral element uptake for better plant health. SeO_4^{-2} exerted strong synergic interactions with S and Mo, whereas SeO_3^{-2} had strong antagonistic interactions with Ca and Mn wheat seedlings. In consideration of the nutrient concentration, plant growth and the interaction with other nutrients for the potential of increasing nutrient contents in grains among these wheat lines, EMB 14, EMB 34, EMB 38 and BRS 264 appeared to be appropriate for a biofortification program with Fe; many lines such as EMB 11, EMB 20, EMB 38 and BRS 264 for Zn; and EMB 10, EMB 14, EMB 38 and BRS 264 for Se.

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Table 1
List of the 20 wheat accessions including 15 varieties and 5 cultivars in the study.

Varieties	Parents	Cultivars	Parents
EMB 1	BRS 207 / CPAC 91161	Brilhante	Brilhante = PF 8640 / BR 24
EMB 5	CPAC 8947 / CPAC 8886	BRS 207	Seri 82 / PF 813
EMB 7	Brilhante / CPAC 961114	BRS 254	BRS 264*3 / ANA 75
EMB 9	BRS 207 / CPAC 9548	BRS 264	Buck Buck / Chiroca // TUI
EMB 10	BRS 207 / CPAC 9548	Supera	PF 9099 / OR 1
EMB 11	CPAC 93175 / Graneiro Inta		
EMB 14	CPAC 93175 / Graneiro Inta		
EMB 15	CPAC 93175 / Graneiro Inta		
EMB 19	BRS 207 / TB 951		
EMB 20	IAPAR 17 / BRS 264		
EMB 26	CPAC 96306 / CPAC 9985		
EMB 30	CPAC 96306 / CPAC 9985		
EMB 33	PF 81627 / BRS 264 // PF 990607		
EMB 34	Taurum/ BRS 207 // PF 8190 / BR 18		
EMB 38	PF 013452 / BRS 207		

[#]The varieties that have the same parents represent different lines selected from F2 crossing.

Table 2

Overview of interactions between elements in the wheat lines and their increase/decrease percentages.

Treatment	Nutrient Interactions										
	P	K	Ca	Mg	S	B	Cu	Fe	Mn	Mo	Zn
Fe	-	-	½I	½I	-	-	-		-	-	-
Zn	-	-	½ I	-	-	-	-	D	½ I	-	
SeO_4^{-2}	-	-	I	I	I*	-	-	I	½ I	I*	½I
SeO_3^{-2}	-	-	D*	½D	D	-	-	-	D*	½D	D
	Nutrient interactions (average %)										
Fe	3.8	8.3	17.0	12.7	3.1	-4.0	4.5	39.9	-8.2	-2.2	8.7
Zn	-1.0	4.7	10.0	-2.5	9.3	1.8	-7.2	-19.2	23	12.1	154.7
SeO_4^{-2}	-5.8	0.2	21.5	18.6	199.7	0.6	5.7	23.8	21.3	71.3	29.3
SeO_3^{-2}	3.4	-1.4	-33.3	-10.6	-23.2	2.1	9.7	5.5	-43.1	-14.4	-24.1

(I) Increase level, (D) Decrease level and, (-) no affect.

*strong antagonistic or synergistic interaction was observed in nearly all wheat lines examined.

Negative numbers represents a reduction in the nutrient concentration.

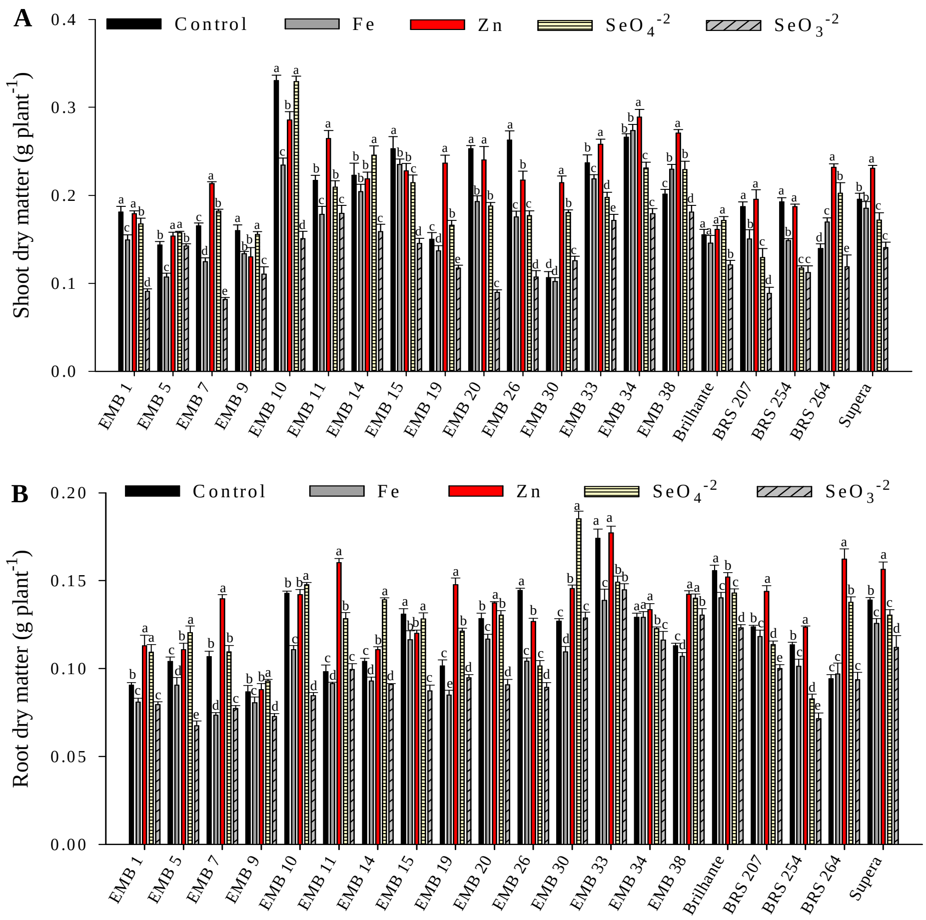
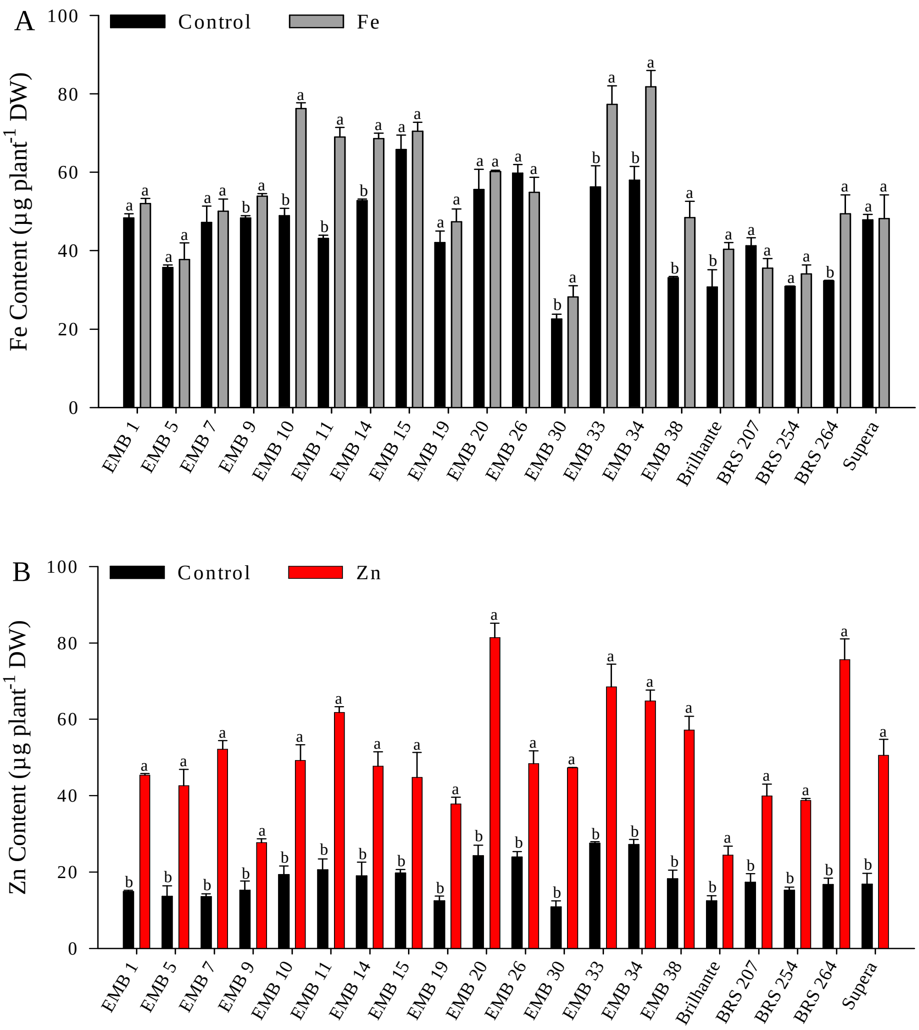


Fig. 1. Effect of Fe, Zn and Se treatments on shoot (A) and root (B) dry matter in seedlings of wheat varieties and cultivars. Values shown are mean ± SE (n=4). Different letters above the column indicate significant difference at $p \leq 0.05$ for treatments.


A
Control
Fe
Fe Content (µg plant-1 DW)
B
Control
Zn
Zn Content (µg plant-1 DW)
0
20
40
60
80
100
EMB 1
EMB 5
EMB 7
EMB 9
EMB 10
EMB 11
EMB 14
EMB 15
EMB 19
EMB 20
EMB 26
EMB 30
EMB 33
EMB 34
EMB 38
Brilhante
BRS 207
BRS 254
BRS 264
Supera

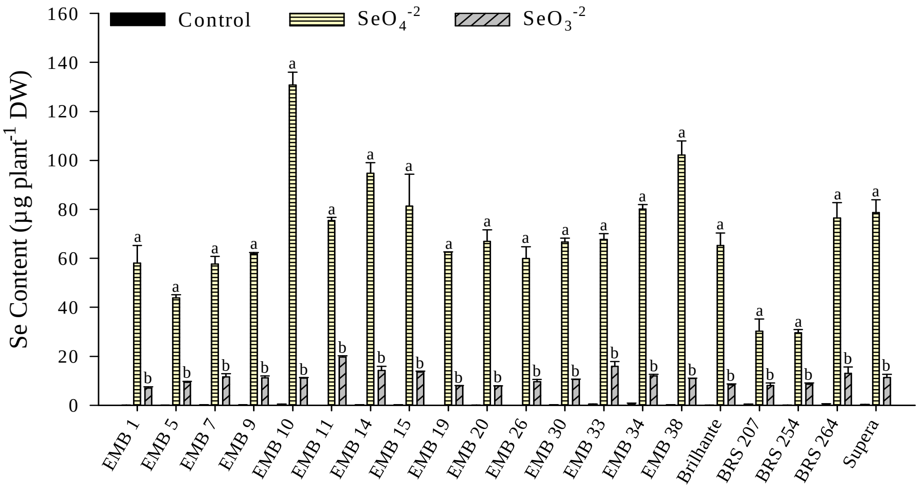
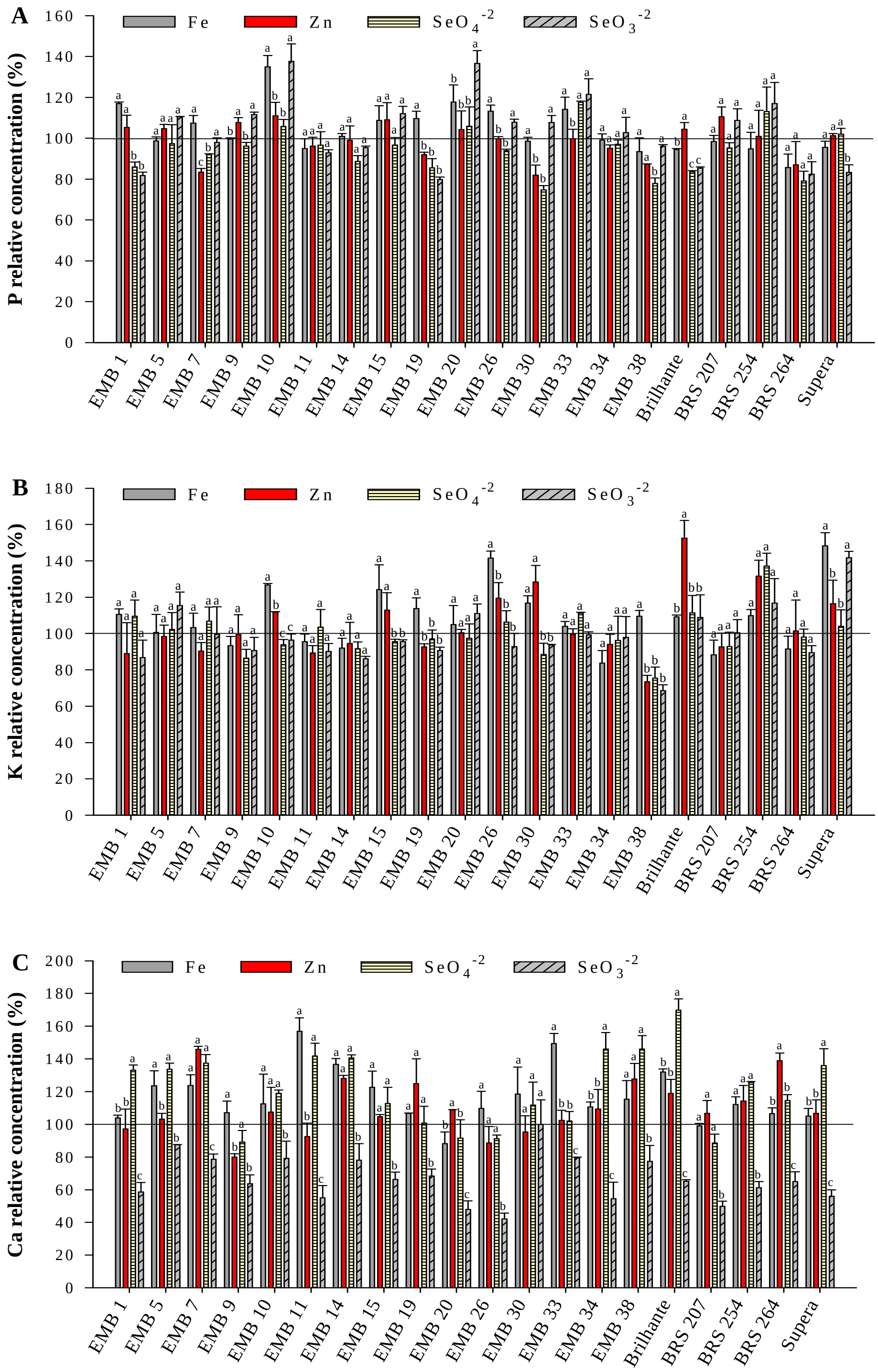
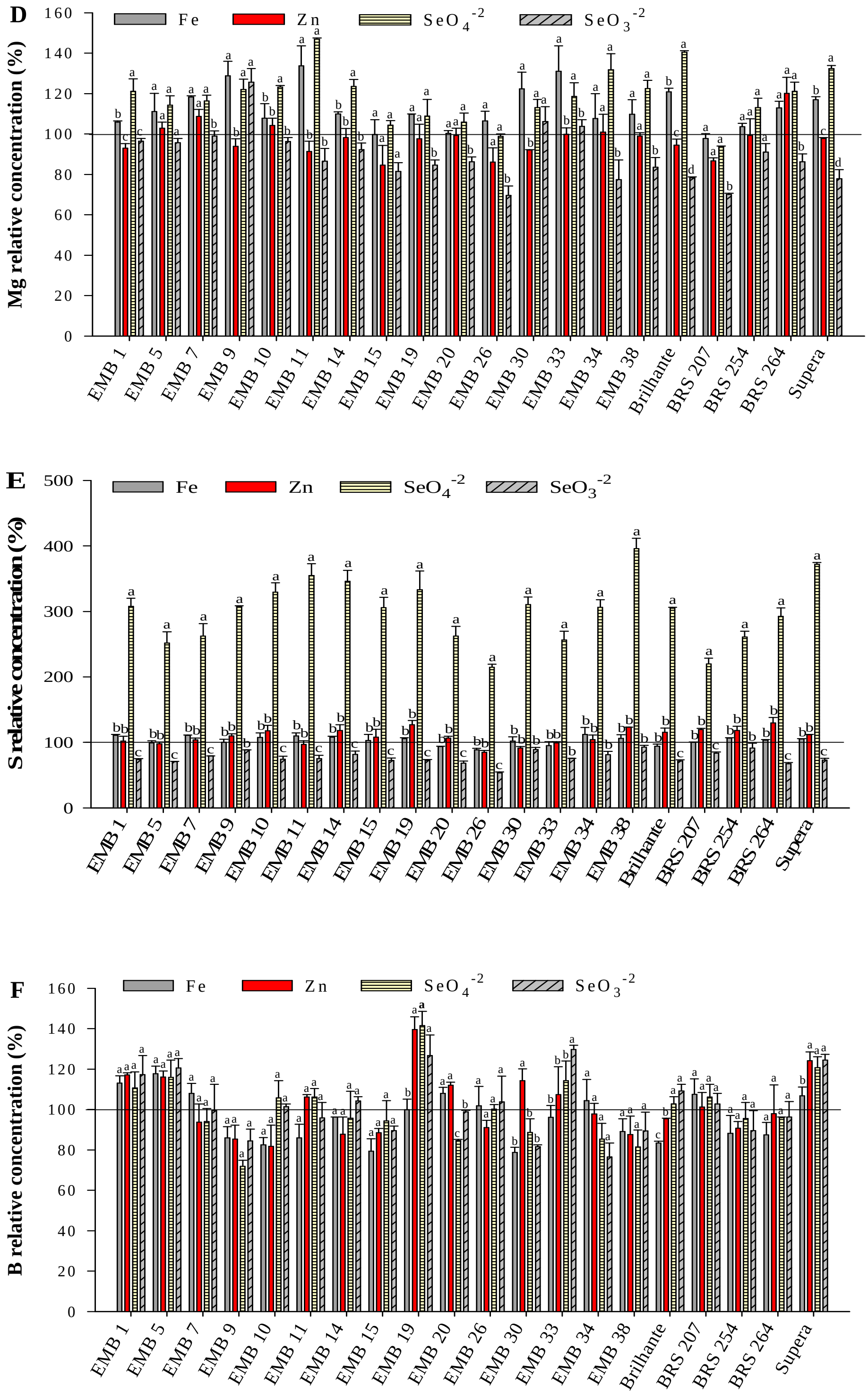
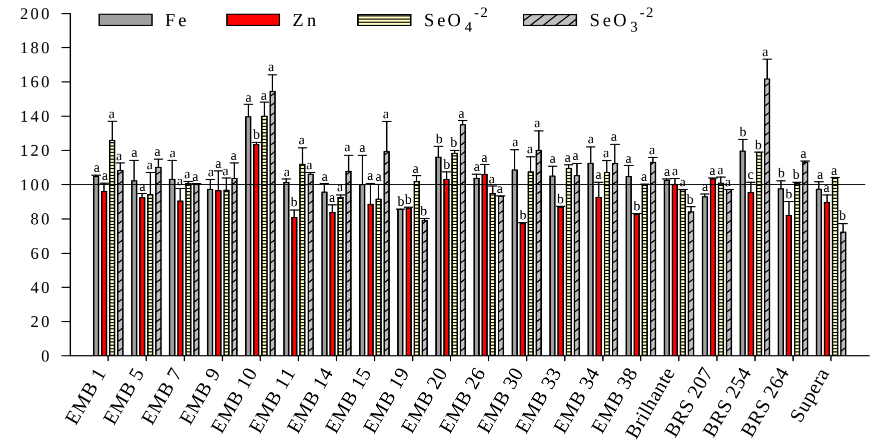
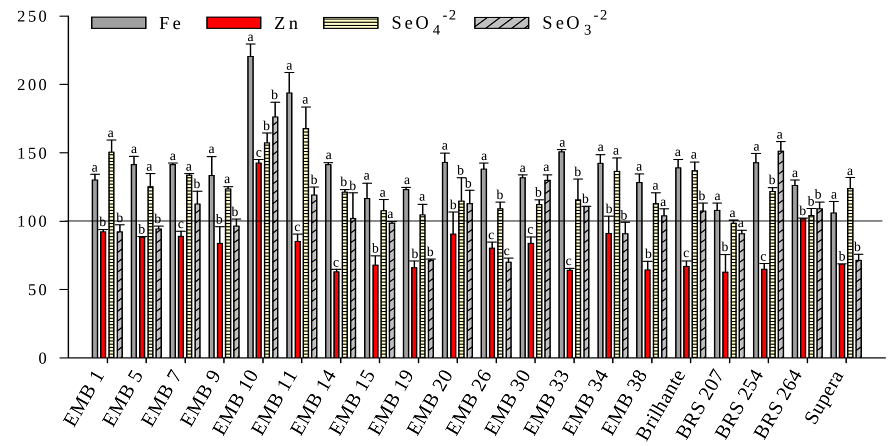


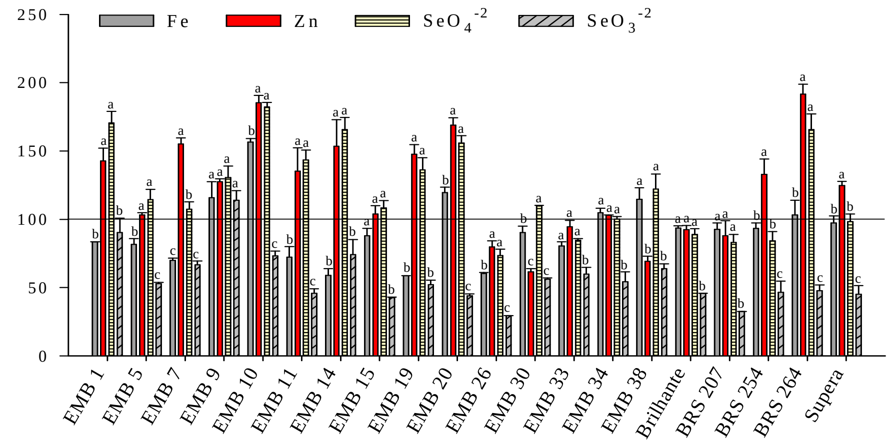
Fig. 2. Effect of Fe, Zn and Se treatments on Fe (A), Zn (B), and Se (C) content in seedlings of wheat varieties and cultivars. Values shown are mean ± SE (n=4). Different letters above the column indicate significant difference at $p \leq 0.05$ for treatments.


A
B
C
Fe
Zn
SeO_4^{-2}
SeO_3^{-2}
P relative concentration (%)
K relative concentration (%)
Ca relative concentration (%)
EMB 1
EMB 5
EMB 7
EMB 9
EMB 10
EMB 11
EMB 14
EMB 15
EMB 19
EMB 20
EMB 26
EMB 30
EMB 33
EMB 34
EMB 38
Brilhante
BRS 207
BRS 254
BRS 264
Supera


D
Fe
Zn
SeO4-2
SeO3-2
Mg relative concentration (%)
0
20
40
60
80
100
120
140
160
EMB 1
EMB 5
EMB 7
EMB 9
EMB 10
EMB 11
EMB 14
EMB 15
EMB 19
EMB 20
EMB 26
EMB 30
EMB 33
EMB 34
EMB 38
Brilhante
BRS 207
BRS 254
BRS 264
Supera
E
Fe
Zn
SeO4-2
SeO3-2
S relative concentration (%)
0
100
200
300
400
500
EMB 1
EMB 5
EMB 7
EMB 9
EMB 10
EMB 11
EMB 14
EMB 15
EMB 19
EMB 20
EMB 26
EMB 30
EMB 33
EMB 34
EMB 38
Brilhante
BRS 207
BRS 254
BRS 264
Supera
F
Fe
Zn
SeO4-2
SeO3-2
B relative concentration (%)
0
20
40
60
80
100
120
140
160
EMB 1
EMB 5
EMB 7
EMB 9
EMB 10
EMB 11
EMB 14
EMB 15
EMB 19
EMB 20
EMB 26
EMB 30
EMB 33
EMB 34
EMB 38
Brilhante
BRS 207
BRS 254
BRS 264
Supera

G

Cu relative concentration (%)
Fe
Zn
SeO_4^{-2}
SeO_3^{-2}
EMB 1
EMB 5
EMB 7
EMB 9
EMB 10
EMB 11
EMB 14
EMB 15
EMB 19
EMB 20
EMB 26
EMB 30
EMB 33
EMB 34
EMB 38
Brilhante
BRS 207
BRS 254
BRS 264
Supera

H

Fe relative concentration (%)
Fe
Zn
SeO_4^{-2}
SeO_3^{-2}
EMB 1
EMB 5
EMB 7
EMB 9
EMB 10
EMB 11
EMB 14
EMB 15
EMB 19
EMB 20
EMB 26
EMB 30
EMB 33
EMB 34
EMB 38
Brilhante
BRS 207
BRS 254
BRS 264
Supera

I

Mn relative concentration (%)
Fe
Zn
SeO_4^{-2}
SeO_3^{-2}
EMB 1
EMB 5
EMB 7
EMB 9
EMB 10
EMB 11
EMB 14
EMB 15
EMB 19
EMB 20
EMB 26
EMB 30
EMB 33
EMB 34
EMB 38
Brilhante
BRS 207
BRS 254
BRS 264
Supera

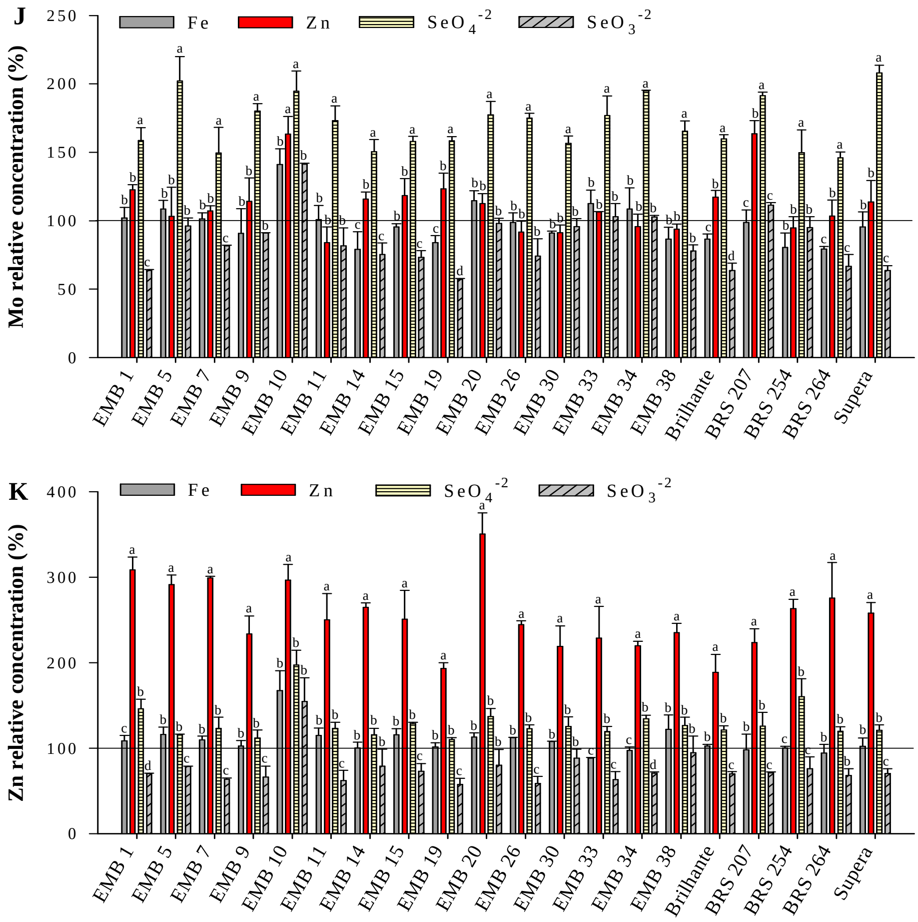


Fig. 3. Relative concentration of nutrients in wheat seedlings of 20 lines in response to Fe, Zn, and Se treatments. The relative nutrient concentrations were calculated by dividing the mineral contents following nutrient treatment with those in control solution. P (A), K (B), Ca (C), Mg (D), S (E), B (F), Cu (G), Fe (H), Mn (I), Mo (J) and Zn (K). Values shown are mean ± SE (n=4). Different letters above the column indicate significant difference at $p \leq 0.05$ for treatments.

Artigo 2

Evaluation of zinc and selenium supplementation and their interaction in wheat plants and grain

Artigo apresentado na íntegra, conforme submetido à revista Journal of Agriculture and Food Chemistry.

Evaluation of zinc and selenium supplementation and their interaction in wheat plants and grain

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Abstract

Humans require many micronutrients for metabolism maintenance and these can be provided in an appropriated diet. The low Zn dietary intake and poor dietary diversity are reported as the mainly reasons for this micronutrient malnutrition in human. Zn and Se are not only crucial for human, but also exert profound effects on plant growth and development. In this study, we selected 20 wheat (*Triticum aestivum* L.) accessions and evaluate genotypic in response of enhancements in Zn and Se in culture solution. Plant micronutrient status affects plant growth and potentially the yield, as well as nutritional quality in edible parts. Taking into consideration the agronomic parameters, nutrient content, and potential of increasing nutrients in these wheat lines we indicate the accessions BRS 207; BRS 254; BRS 264; EMB 19 and EMB 33 for biofortification with Zn; many lines such as Brilhante; BRS 264; EMB 7; EMB 26 and EMB 30 appeared to be appropriate for Zn+SeO_4; and Brilhante; BRS 254; Supera; EMB 20 and EMB 33 for Zn+SeO_3.

Keywords: Selenate, selenite, biofortification, nutrient content, *Triticum aestivum L.*

1. Introduction

Micronutrient malnutrition is a well-known human health problem, which justifies any effort concerning food enrichment with such elements. Nowadays, nearly 3 billion people worldwide are affected by micronutrient deficiencies, including iron (Fe), zinc (Zn), iodine (I), and selenium (Se) [1–3]. Earlier agricultural programs, e.g., the 1960´s Green Revolution, have focused on increasing grain production, without paying attention to nutrient and vitamin contents in most crops. Consequently, the new challenges for agriculture in the world are quality and safety of staple food [3–5]. Genetics and physiological factors determine the nutrient uptake and consequently the productivity and quality, yet these factors are likely to change among plant species, cultivars, tissues and organs [3,6]. Some studies suggest that it is possible to increase the nutrient content in plants while maintaining high plant yields [6–8]. Moreover the nutritional value of many crops can be increased via mineral fertilization with important nutrients such as Zn, Se, copper (Cu) and nickel (Ni)[9]. In fact, improving the micronutrients content of many important crops using different biofortification strategies has been the subject of many studies [7,8,10].

Zinc (Zn) is an essential element for plants, humans and animals. This element plays roles in over 300 enzymes and is a cofactor in thousands of proteins, including transcription factors [11–13]. At least 50% of grains areas in the world are under Zn-deficient soils, and Zn status in plants are correlated with plants growth, nutritional quality and crop yield[8,14]. Germplasms have different capacities to accumulate nutrients showing the possibility to enhance staple food quality with some essential elements [10]. In wheat grains, at least a 2-fold variation in Zn concentration have been observed [7,15]. However, Zn concentration in cultivated wheat is lower than primitive and wild wheat lines, suggesting more potential for plant breeding [8].

Selenium (Se) is an essential element for humans and animals and also an important component of antioxidant systems [15,16]. In plants, Se has shown beneficial effects [17], but the essentiality is not determinate. Plants uptake Se from soils mainly as selenite (SeO_3^{-2}) and selenate (SeO_4^{-2}). Selenate is actively transported in a process mediated by sulfate transporters while selenite is passively transported and it is believed that phosphate transporters preside over the process [18–20]. Wheat grains cultivated in different Australian soils showed 144-fold variation in Se concentration, but much of this variation was associated with spatial variation in soil selenium, but much of this variation was associated with spatial variation in soil selenium [15,21].

Wheat is the major staple crop that provides approximately 30% of food source for a large number of people worldwide. Plants with increased ability to acquire mineral elements improve their growth, and consequently increase yield as well as mineral concentrations in edible grains [3,10]. From the wheat breeding program at Embrapa Cerrados CPAC, Planaltina, Brazil, we selected 20 wheat accessions to evaluate the genotypic variation and nutrient interaction in response to Zn and Se supplements. The effects of Zn and Se fertilizations on wheat grains and nutrient content were investigated in order to provide the information for selection of wheat accessions with substantial capacity to acquire minerals and for crop biofortification with these micronutrients. Interactions among nutrients are known to impact plant nutrient status, plant health and yield [22,23]. The effects of Zn and Se treatments on other nutrient accumulations in these wheat germplasms were also examined to provide a general view of mineral interactions in wheat plants.

2. Materials and methods

2.1 Wheat germplasm and plants growth

For this study a population of bread wheat (*Triticum aestivum* L.) was selected from the Embrapa Cerrados breeding program (Planaltina, GO, Brazil). This population included 20 accessions with 15 varieties and 5 cultivars (Table 1), which are important cultivars and lines with potential to grown in Brazil.

Seeds of each wheat variety or cultivar were surface-sterilized in 0.5% NaOCl for 15 minutes, rinsed in distilled milli-Q water, and germinated in a chamber at 18^{o}C for 3 days on moistened filter paper. The 3-day-old young seedlings with roots of approximately 3 cm long were transplanted into 10.0 L pots containing nutrient solution. The nutrient solution consisted of 1 mM KNO_3, 1 mM $Ca(NO_3)_2$, 0.05 mM $NH_4H_2PO_4$, 0.25 mM $MgSO_4$, 0.1mM NH_4NO_3, 12.5 µM H_3BO_3, 0.1 µM Na_2MoO_4, 0.1 µM $NiSO_4$, 0.4 µM $MnSO_4$, 1.6 µM $CuSO_4$, 96 µM $Fe(NO_3)_3$, 10 µM $ZnSO_4$, 128 µM H_3HEDTA, and 2 mM MES buffer (pH 6.0). $ZnSO_4$ and $Fe(NO_3)_3$ were equilibrated with H_3HEDTA (N-2-hydroxyethylethhylenediamine-N,N',N'-triacetic acid) before adding to the nutrient solution. The H_3HEDTA was used as micronutrient buffer [24].

Wheat plants were grown with aeration in a greenhouse at 15-20 ºC under 16-h day length. When plants started blooming, the treatments were applied. These plants were either grown in nutrient solution (Control) or exposed to the nutrient solution containing, 50 µM $ZnSO_4$ (Zn), 50 µM $ZnSO_4$ + 10 µM Na_2SeO_4 (Zn+SeO_4) and 50 µM $ZnSO_4$ + 10 µM Na_2SeO_3 (Zn+SeO_3). The speciation program GEOCHEM® was used to calculate the solution activity. The nutrient solution was changed every week. A total of 240 plants (20 lines x 4 treatments x 3 replicates) were harvested at maturity and washed in distilled water. Shoots (including glume), roots and grains were separated and dried at 65ºC for 72h. Flag leaves were collected to determinate the mineral content in shoots.

2.2. Nutrients content in flag leaves and grains

Total mineral contents in the samples were determined using an inductively coupled plasma-emission spectrometer (model ICAP 61E trace analyzer, Thermo Electron, San Jose, CA). Individual dried flag leaves (200 mg) and grain (500 mg) samples were weighed and predigested with 4 mL (10 mL) of concentrated $HNO_3/HClO_4$ (60/40% v/v) at room temperature overnight. The samples were then heated to 120ºC for 2 hours, followed by 195ºC for 30 min. The samples were cooled at room temperature and diluted with milli-Q water to 20 mL. All samples were analyzed in triplicate. Blank and an internal standard yttrium were used to substantiate the accuracy of the analytical results obtained. After that, we calculated the Zn efficiency of lines to shoot and grain dry matter and content under Zn normal (control) to Zn addition (50 μM). We also calculated the Zn accumulation (%) to grains and shoots of these wheat accessions.

The experimental samples were prepared and analyzed following a rigid quality assurance/quality control (QA/QC) to ensure accurate and reliable analytical data.

2.3. Statistical analysis

All quantitative data on plant growth and mineral nutrient contents were subjected to statistical testing by one way analysis of variance (ANOVA). The data were submitted variance analysis by the Scott-Knott test or Test F, for Se content, at a level of 5% of probability, using the statistical software SISVAR [25].

3. Results and Discussion

3.1. Grains production

Micronutrients, such as Zn and Se, are not only crucial for humans, but also wield intense effects on plant development [3,9,10]. To examine the responses of Zn, Se and their interaction in these selected germplasms, individual parts of each wheat line were separately harvested and died and their biomasses measured.

These wheat lines showed up to 4-fold differences in their grain production when comparing the treatments applied in the nutrient solution (the values ranged from 6.1 to 31.3, $p \leq 0.05$) (Figure 1A). When these wheat lines were treated with additional Zn, 50 µM, a general tolerance was observed in most of the lines. Enhancements were observed of up to 37% in these lines. However, four lines (EMB 15, EMB 30, BRS 207 and BRS 264) appeared not to tolerate Zn supplementation and showed a slight reduction in their grain production. Zn fertilization has been shown to enhance plant growth and grain yield, along with an increase in Zn bioavailability in grains [8,26,27]. Although Se is considered to be non-essential for plants, Se at low dosages promotes plant growth [28,29]. When plants were treated with additional Zn and different forms of Se, singular responses were noticed. Grain production in wheat lines grown in culture solution with selenate in the presence of Zn ($Zn+SeO_4$) showed a general suppression in comparison with those plants that received 50 µM Zn (Fig. 1A). Nevertheless, in 3 wheat lines (EMB 30, BRS 207 and BRS 264) enhancements were observed in grain yield at an average of 12%, 90% and 23%, respectively. Previous studies have reported genetic variations among crop germplasm in response to SeO_4 treatment [30]. A response pattern similar to $Zn+SeO_4$ was observed for wheat plants exposed to Zn and selenite ($Zn+SeO_3$) ($p \leq 0.05$, Fig. 1A). However, a general reduction was observed in comparison with Zn-treated plants, showing that selenite is more toxic than selenate, which is in accordance with results reported previously [18,28].

The grains weight of 100 seeds in response of these wheat lines to Zn and Se supplementation were also researched and a genetic variation in these germplasms was observed (Figure 1B). When wheat plants were treated with Zn, most of the lines responded positively or were unaffected as to their grain weight of 100 seeds. A 21% average weight increase was observed. These results are in agreement with those reporting yield increases following Zn fertilization [8,27]. Most of the wheat lines treated with $Zn+SeO_4$ showed a decrease in the grain weight of 100 seeds. However 3 lines (EMB 5, EMB 30 and BRS 264) presented enhancements, an average of 14%, when SeO_4 was applied with Zn. A previous work showed no effect on production yield in winter wheat receiving liquid or granular Se fertilization[31] diverging from the results found in this present work. Generally, grain weights were not affected when $Zn+SeO_3$ was applied in nutrient solution. Nevertheless, 5 lines (EMB 5, 15, 19, 26 and 30) increased the grain weight of 100 seeds, an average 16%. These results suggest that Zn increases the number of grains and, despite Se being more toxic and reducing the grain production, when applied with Zn it makes the grains heavier.

3.2. Different capacity of wheat accessions to concentrate nutrients in shoots (flag leaves)

The total nutrient content was investigated in wheat leaves in response to the addition of Zn and different forms of Se, selenate and selenite application. Wheat plants exposed to treatments showed different capacity to concentrate minerals in shoots (flag leaves) (Figure 2). When additional Zn was applied in culture solution, the Zn content in leaves increased in all lines (Fig. 2A). The Zn level increase observed was up to 4-fold, implying a high capacity of these lines to take in and accumulate Zn. Increases in Zn concentration following Zn addition were observed in wheat seedlings and potato [32,33]. SeO_4 in presence of additional Zn did not affect, enhanced or suppressed the Zn content in wheat leaves, showing the different capacity of these lines to uptake and assimilate Se [18,19]. However, some lines (EMB 1, EMB 14, EMB 19 and BRS 264) increased the Zn content in the flag leaves up to 1-fold when SeO_4 was supplied in nutrient solution. Plants exposed to $Zn+SeO_3$ showed a general inhibition of Zn content, exhibiting the highest selenite toxicity. These results agree with those reported in previous works [33,34]. Despite this evidence, some lines increased Zn content in leaves and, the cultivar Brilhante enhanced the Zn concentration to over 4-fold. Differences in Fe, Zn, Cu, Mn and Se concentrations and mineral localization in hard red wheat genotypes and *Triticum* species have been reported previously [5,35,36].

Se was not detected in shoots (flag leaves) of wheat lines that grew under the Control and Zn in nutrient solution treatments (Fig. 2B). However, when these wheat lines were exposed to SeO_4 and SeO_3 with additional Zn, the total Se content exhibited different capacity to concentrate Se, while the SeO_4-treated showed increases in Se content up to 1-fold, plants SeO_3-treated enhanced up to 2-fold. Wheat plants exposed to SeO_4 contained over 41-fold more than SeO_3-treated wheat plants, consistent with previous reports that plants accumulate more Se when treated with SeO_4 than SeO_3 [18,28,34]

The interactions and effects on nutrient content are well-documented and Zn is known to influence P assimilation and uptake [16,22] as is reported for Se and S [19]. The P content in wheat plants was relatively consistent among wheat lines in the Control treatment (Fig. 2C). However, Zn-treated plants showed unaffected, increased or inhibited P content. These results are in agreement with those reported for potatoes [32]. On the other hand, a general inhibition of P content in wheat leaves was observed in Se-treated plants. SeO_3 had lower effect on P content than SeO_4, suggesting a possibility of phosphate transporters in this process [18,19].

Sulfur content was rather consistent among wheat lines grown in culture solution and had a response pattern similar to P content when they were exposed to Zn. However, when SeO_4 was applied, S content increased for all wheat accessions to over 12-fold, exhibiting a strong stimulation of S by selenate at the dose added. A 3-fold variation in S levels was observed in these wheat accessions. S deficient wheat plants increased the Se content in grains up to 6.4-fold [37]. By contrast, the S contents were reduced in nearly all wheat accessions when the plants were SeO_3-treated. These results confirm that Se forms imposed opposite effects on S content in wheat plants, as shown in other studies [18,28].

3.4. Different capacity of wheat accessions to concentrate nutrients in grains

To investigate the responses of wheat lines to additional Zn as well as to different Se forms, the total mineral content in wheat grains were determined. The wheat accessions showed different capacity to concentrate Zn when they grew in culture solution (Fig. 3A). The Zn supplementation increases the Zn content in all wheat lines. Increases in Zn concentration following Zn fertilization were observed in wheat [26,27]. When SeO_4 was added in the culture solution, the plants were unaffected, increased or suppressed as to the Zn levels ($p \leq 0.05$). An average increase of 27% in Zn concentration was observed. On the other hand, an inhibition of Zn concentration was detected in almost all accessions of wheat grains SeO_3-treated, except lines EMB 9 and EMB 20. These results confirm the differences among lines, pointing out the genotypes that could be used in plant breeding, taking into account specific Zn target contents, e.g., that establish by HarvestPlus Program at 33 mg Zn kg^{-1} (www.harvestplus.org).

Se was undetected in wheat grains under Control and Zn treatments in culture solution and showed a 2.4-fold difference between lines with high and low Se levels in Se-treated plants (Fig. 3B). In general, plants accumulate more Se when SeO_4 is added in nutrient solution than SeO_3. The small differences between SeO_3 and SeO_4 found in this study agree with those reported in rice Se-treated grains [34]. However a high suppression occurs in grains in comparison with leaves (Fig. 2B), agreeing with those results found for rice and wheat [34,38]. By contrast, Se levels that were analyzed in cultivated spring wheat crop showed grain Se content higher than shoots [39,40]. Broadley et al.[31] reported that 10 g Se ha^{-1} could increase 10-fold the Se concentration in wheat grain.

Phosphorous content in wheat grains was relatively consistent among wheat lines in culture solution. Zn-treated wheat plants were unaltered or showed a slight suppression in grain P levels for nearly all accession except cultivars BRS 254 and BRS 264 ($p \leq 0.05$) (Fig. 3C). Interactions between these two elements have been reported in the literature [16,22]. When Se was applied in nutrient solution with additional Zn, a general inhibition was observed. While SeO_4 did not affect or it inhibited P content in some lines, the SeO_3-treated increased in P content (EMB 7, EMB 9, EMB 20 and Supera, $p \leq 0.05$). These results suggest that SeO_4 is more toxic than SeO_3 to P levels, confirming the results found for leaves in which phosphate transporters act on this process [18,19].

A similar pattern of P content result was found for S levels in wheat plants for all treatments (Fig. 3D). While SeO_4 exhibited enhancements, did not affect or resulted in a low suppression, SeO_3 showed a general inhibition. These results confirm the affinity of selenate with sulphate transports [18,19].

3.3. Zn efficiency of wheat accessions Zn and Se-treated

To evaluate the Zn efficiency we considered the treatment control as Zn deficient. In this study, an extreme genetic diversity was found for Zn efficiency ratios, which showed different responses to the lines and treatments. Apparently, SeO_3-treated plants showed the biggest mean values for shoot and grain dry matter and Zn concentration. Shoots of wheat SeO_3-treated plants improved the Zn efficiency and, under this treatment the highest means values of 100%, 96% and 129%, respectively were observed, for Zn, Zn+SeO_4 and Zn+SeO_3 (Table 2). The line EMB 20 was more efficient in using Zn, however this line showed the lowest shoot dry matter production value, 31% in comparison with the control (data not shown). These results are similar to those reported for diploid, tetraploid and hexaploid wheat [41]. A response pattern similar to shoots was observed for Zn efficiency in grain production, while Zn and Zn+SeO_4 showed mean values of 88% and 109%, the Zn+SeO_3 treatment showed 114%. In spite of the higher values for Zn efficiency, the lines of the Zn+SeO_3 treatment that showed highest in Zn efficiency, showed the lowest values for grain production. Wheat experiments, in Zn-deficient soils, showed seed Zn content enhancements, however the high Zn efficiency was not associated with high dry matter or Zn efficiency [42].

The Zn efficiency for nutrient concentration in shoots and grains has a similar pattern as the abovementioned (Table 3). However, in 50 μM Zn, the line having the highest Zn efficiency was EMB 33. When we evaluated the grain concentrations, the most efficient Zn use line was EMB 11 Zn+SeO_3-treated, which showed the lowest Zn concentration values. Despite SeO_3 reducing the shoot and grain production, the highest Zn efficiency was observed under this treatment.

3.4. Interaction among nutrients in wheat grains Zn- and Se-treated

To examine the effect of Zn and Se addition in wheat grains, cultivated in culture solution upon the accumulation of the other minerals, we measured treatment responses in terms of the relative concentration of a number of minerals (Supplementary Figure S1). Adding the treatments in culture solution resulted in a slight reduction in P concentration in all wheat lines (Fig. S1A), which agrees with similar results reported for wheat seedlings in culture solution [33]. The P-Zn interaction is well-known and documented and Zn supplementation decreases P levels in grains and vice-versa [16,22]. In spite of the P-Zn interaction, the treatment Zn+SeO_4 showed the lowest values for relative P concentration in almost all lines. On the other hand, this treatment increased the Zn concentration in the grains, which explains the P reduction due to the antagonism between these nutrients [16,22] (Fig. 3A). In general, K concentrations (Fig. S1B) and Ca (Fig. S1C) were not affected by the treatments. The relative concentration of Mg (Fig S1D) in wheat grain was similar to those reported for P. Sulfur concentration the grain showed different response in each treatment; while half of the lines Zn- and Zn+SeO_4-treated showed a small enhancement in the relative concentration of S, Zn+SeO_3 reduced S concentration in all lines, with the exception of EMB 30 (Fig. S1E). S and Se have a synergic interaction [28,33], but Se added with Zn was not sufficient to increases the S content in grains. When we examine micronutrients, they showed different responses in each treatment. Boron (Fig. S1F) was not affected by Zn or Se addition, whereas the nutrients Cu (Fig. S1G), Fe (Fig. S1H), Mn (Fig. S1I) and Mo (Fig. S1J) had suppression in their relative concentration when the treatments were applied. The treatment Zn+SeO_3 was apparently more toxic to Mn levels in wheat plants. In general, Zn supplementation increases Zn concentration in all treatments and lines (Fig. S1K). A two-fold increase was observed in some lines. Apparently, some lines, like EMB 5; 7; 9; 30 and BRS 264, were stimulated with SeO_4 addition, showing different germplasm responses and, the possibility to work these elements together. On the other hand, SeO_3 decreases Zn concentration in most lines.

Conclusions

The treatments affected the mineral status and quality, growth and yield in wheat tissues. These wheat lines showed genotypic variations in response to Zn, Zn+SeO_4 and Zn+SeO_3 treatments exhibiting differences in Zn efficiency. Thus, the study will be useful to develop and select cultivars with better plant ability to concentrate micronutrients in edible parts. Taking into consideration the agronomic parameters, nutrient content, and potential of increasing nutrients in these wheat lines we indicate the accessions BRS 207; BRS 254; BRS 264; EMB 19 and EMB 33 for biofortification with Zn; many lines such as Brilhante; BRS 264; EMB 7; EMB 26 and EMB 30 appeared to be appropriate for Zn+SeO_4; and Brilhante; BRS 254; Supera; EMB 20 and EMB 33 for Zn+SeO_3.

Abbreviations

Fe, iron; Zn, zinc; Se, selenium; I, iodine; Cu, copper; Ni, nickel; H_3HEDTA, N-2-hydroxyethylethhylenediamine-N,N',N'-triacetic acid; MES, 2-(N-morpholino)-ethane sulfonic acid; Na_2SeO_4, sodium selenate; Na_2SeO_3, sodium selenite; Zn+SeO_4, zinc (50 μM) + selenate (10 μM); Zn+SeO_3, zinc (50μM) + selenite (10 μM); QA/QC, quality assurance/quality control; P, phosphorous; S, sulfur; K, Potassium; Ca, Calcium; Mg, Magnesium; B, Boron; Fe, Iron; Mn, Manganese; Mo, Molybdenum.

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Figure captions

Figure 1. Effect of treatments on grain dry matter (A) and weight of 100 grains (B) in wheat varieties and cultivars. Values shown are mean ± SE (n = 3). Different letters above the column indicate significant difference at $p \leq 0.05$ by Skott-Knott test.

Figure 2. Effect of treatments on Zn (A), Se (B), P (C) and S (D) content in wheat shoots. Values shown are mean ± SE (n = 3). Different letters above the column indicate significant difference at $p \leq 0.05$ by Skott-Knott test.

Figure 3. Effect of treatments on Zn (A), Se (B), P (C) and S (D) content in wheat grains. Values shown are mean ± SE (n = 3). Different letters above the column indicate significant difference at $p \leq 0.05$ by Skott-Knott test.

Supplemental Figure S1. Relative content of nutrients in wheat grains of 20 lines in response to Zn and Zn+Se treatments. The relative nutrient concentrations were calculated by dividing the mineral contents following nutrient treatment with those in control solution. P (A), K (B), Ca (C), Mg (D), S (E), B (F), Cu (G), Fe (H), Mn (I), Mo (J) and Zn (K). Values shown are mean ± SE (n=3). Different letters above the column indicate significant difference at $p \leq 0.05$ for treatments.

Table 1 – List of wheat accessions used in the experiment (15 varieties and 5 cultivars).

Varieties	Parents	Cultivars	Parents
EMB 1	BRS 207 / CPAC 91161	Brilhante	Brilhante = PF 8640 / BR 24
EMB 5	CPAC 8947 / CPAC 8886	BRS 207	Seri 82 / PF 813
EMB 7	Brilhante / CPAC 961114	BRS 254	BRS 264*3 / ANA 75
EMB 9	BRS 207 / CPAC 9548	BRS 264	Buck Buck / Chiroca // TUI
EMB 10	BRS 207 / CPAC 9548	Supera	PF 9099 / OR 1
EMB 11	CPAC 93175 / Graneiro Inta		
EMB 14	CPAC 93175 / Graneiro Inta		
EMB 15	CPAC 93175 / Graneiro Inta		
EMB 19	BRS 207 / TB 951		
EMB 20	IAPAR 17 / BRS 264		
EMB 26	CPAC 96306 / CPAC 9985		
EMB 30	CPAC 96306 / CPAC 9985		
EMB 33	PF 81627 / BRS 264 // PF 990607		
EMB 34	Taurum / BRS 207 // PF 8190 / BR 18		
EMB 38	PF 013452 / BRS 207		

[#]The varieties that have the same parents represent different lines selected from F2 crossing.

Table 2 – Effects of the treatments on the Zn use efficiency (%) of shoot and grain dry matter of wheat accession.

	Dry matter (%)*					
	Shoot			Grain		
Accessions	Zn	$Zn+SeO_4$	$Zn+SeO_3$	Zn	$Zn+SeO_4$	$Zn+SeO_3$
EMB 1	81	103	94	67	94	83
EMB 5	63	91	92	66	99	89
EMB 7	99	110	92	102	121	95
EMB 9	77	121	132	71	134	131
EMB 10	49	69	55	80	121	117
EMB 11	87	61	204	99	96	177
EMB 14	141	137	142	68	89	81
EMB 15	115	92	111	128	157	139
EMB 19	80	94	71	48	82	45
EMB 20	106	129	322	97	114	132
EMB 26	82	136	120	98	154	95
EMB 30	113	105	117	127	114	127
EMB 33	85	94	115	46	65	82
EMB 34	59	54	66	54	95	64
EMB 38	146	92	183	88	109	142
Brilhante	86	91	89	81	120	120
BRS 207	167	68	135	137	75	121
BRS 254	92	55	81	83	96	82
BRS 264	167	101	210	137	111	175
Supera	105	118	142	92	135	187
Mean	100	96	129	88	109	114

*The values are means of 3 replicates.

Table 3 - Effects of the treatments on the Zn accumulation of shoot and grain concentrations of wheat accession.

	Zn accumulation (%)*					
	Shoot			Grain		
Accessions	Zn	Zn+SeO_4	Zn+SeO_3	Zn	Zn+SeO_4	Zn+SeO_3
EMB 1	110	50	40	51	54	69
EMB 5	30	31	56	63	47	75
EMB 7	26	39	64	62	52	98
EMB 9	40	28	43	61	47	62
EMB 10	30	34	83	53	59	80
EMB 11	30	36	116	51	64	116
EMB 14	71	35	41	41	39	58
EMB 15	27	26	74	59	67	68
EMB 19	49	22	83	64	60	94
EMB 20	45	37	52	57	59	62
EMB 26	31	26	51	42	45	73
EMB 30	23	24	31	67	59	81
EMB 33	154	130	108	34	32	41
EMB 34	49	43	74	45	51	72
EMB 38	28	25	26	62	70	85
Brilhante	59	55	11	63	62	82
BRS 207	18	32	24	48	85	77
BRS 254	29	27	77	61	67	75
BRS 264	25	13	26	78	57	72
Supera	25	29	14	63	79	79
Mean	45	37	55	56	58	76

*The values are means of 3 replicates.

Figures

Fig. 1

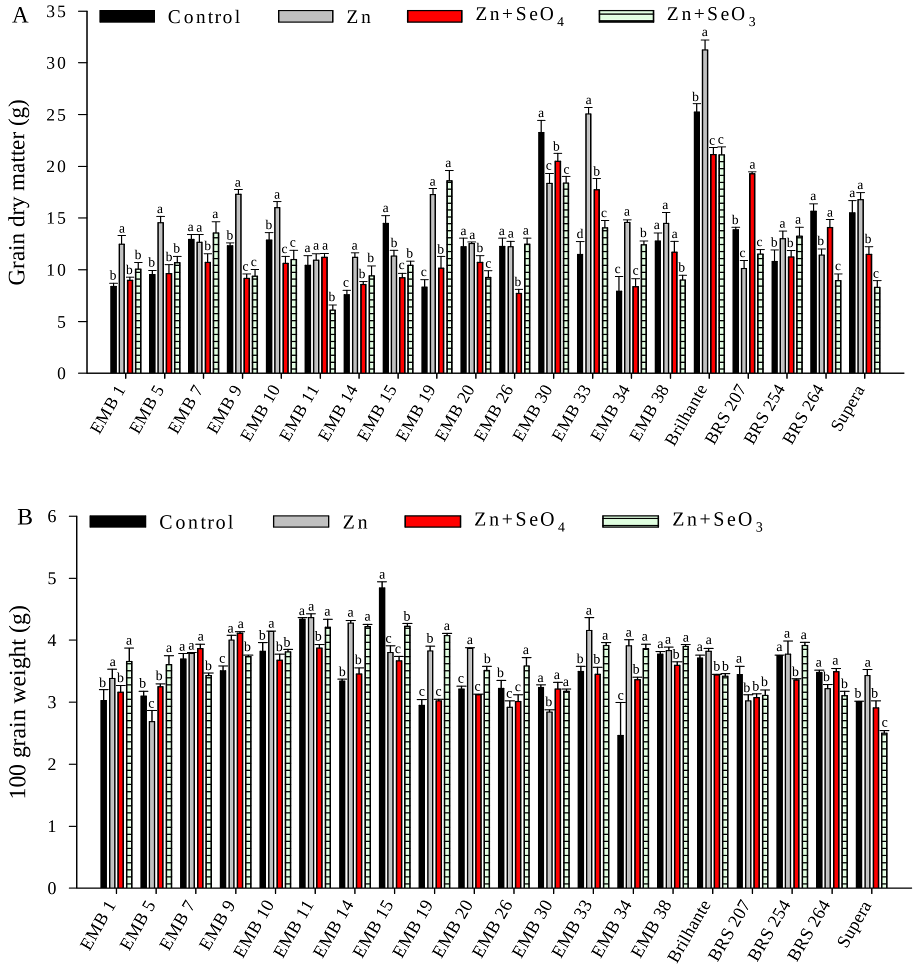
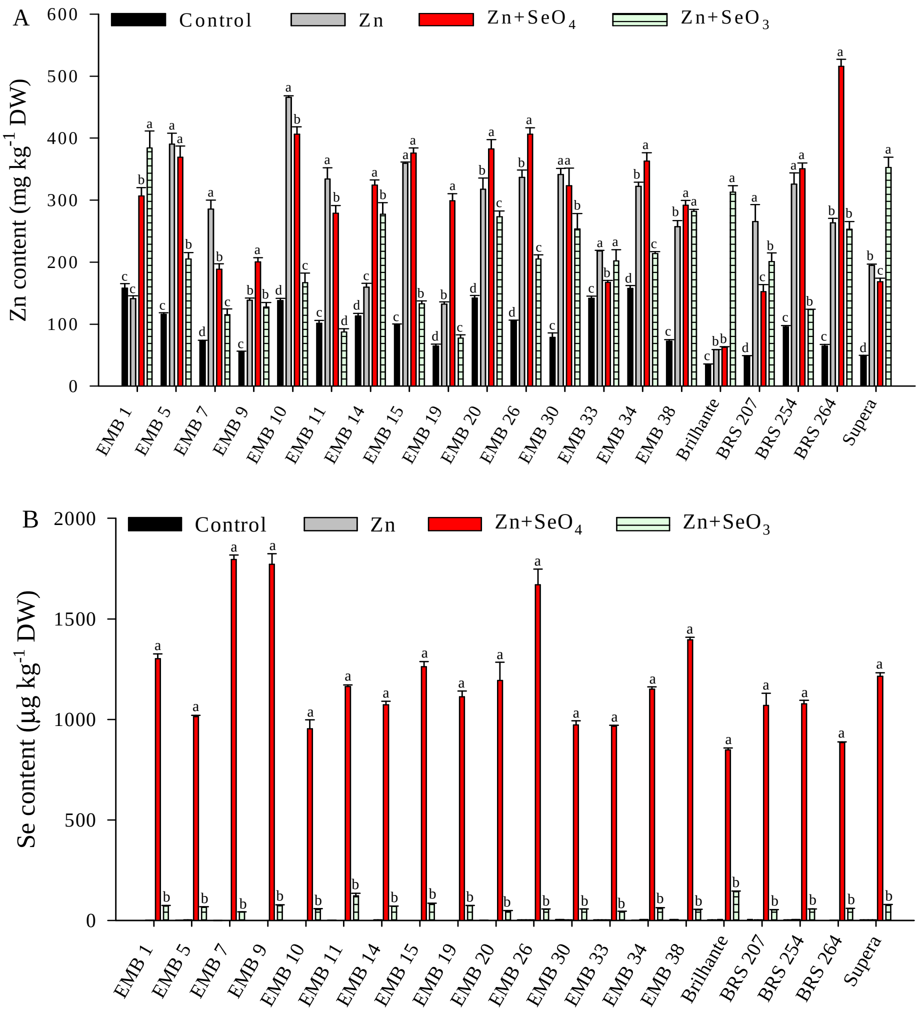


Figure 1. Effects of treatments on grain dry matter (A) and weight of 100 grains (B) in wheat varieties and cultivars. Values shown are mean ± SE (n = 3). Different letters above the column indicate significant difference at $p \leq 0.05$ by Skott-Knott test.

Fig. 2


A
Control
Zn
Zn+SeO4
Zn+SeO3
Zn content (mg kg-1 DW)
0
100
200
300
400
500
600
EMB 1
EMB 5
EMB 7
EMB 9
EMB 10
EMB 11
EMB 14
EMB 15
EMB 19
EMB 20
EMB 26
EMB 30
EMB 33
EMB 34
EMB 38
Brilhante
BRS 207
BRS 254
BRS 264
Supera
B
Control
Zn
Zn+SeO4
Zn+SeO3
Se content (µg kg-1 DW)
0
500
1000
1500
2000

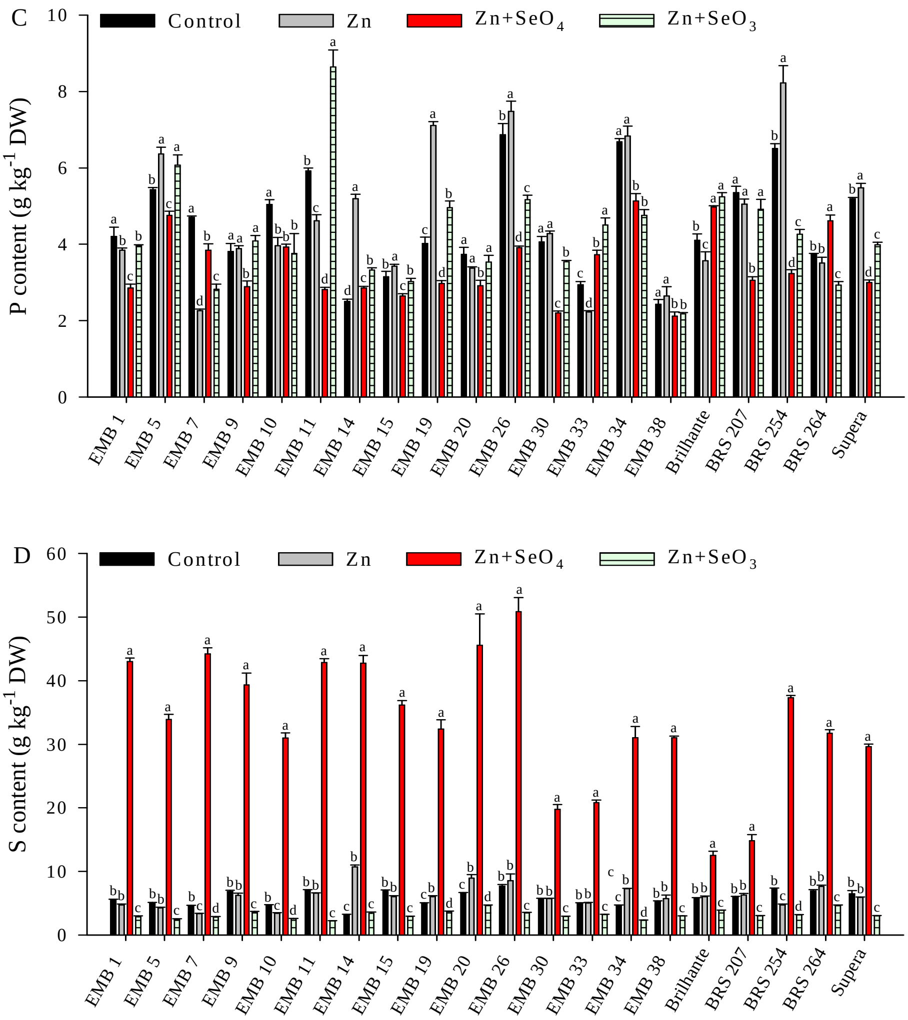
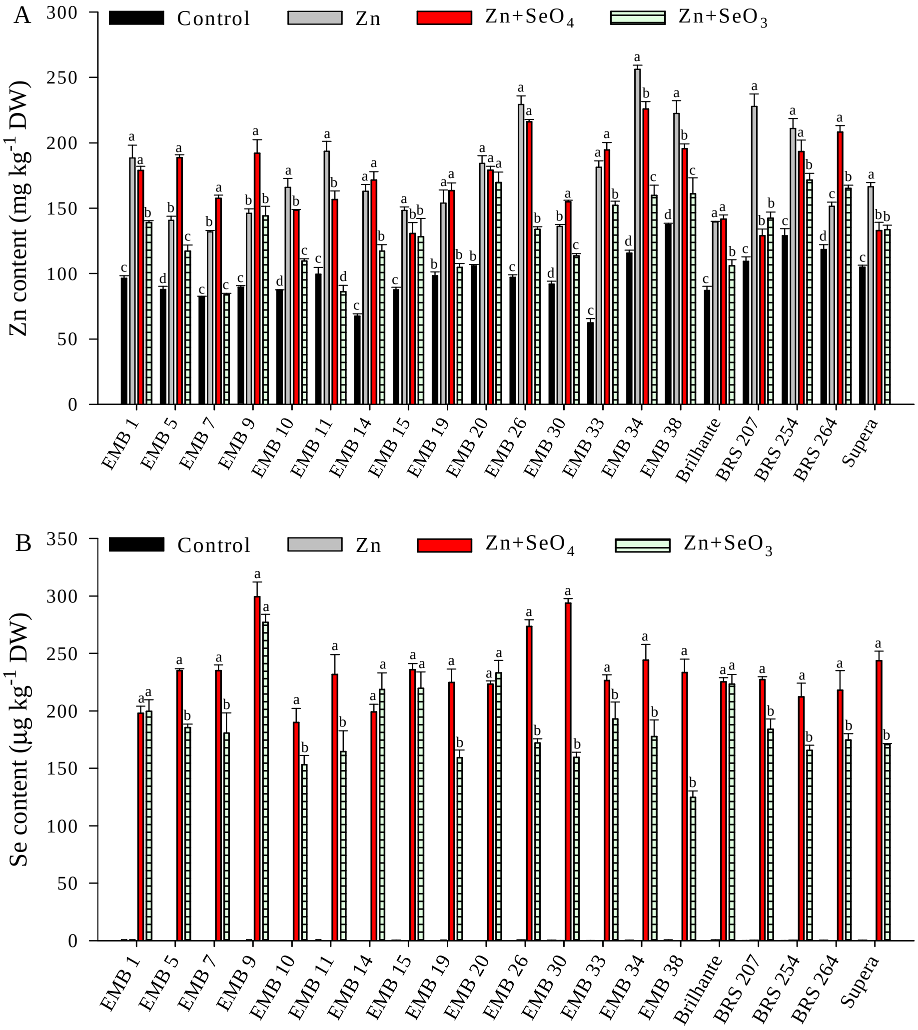
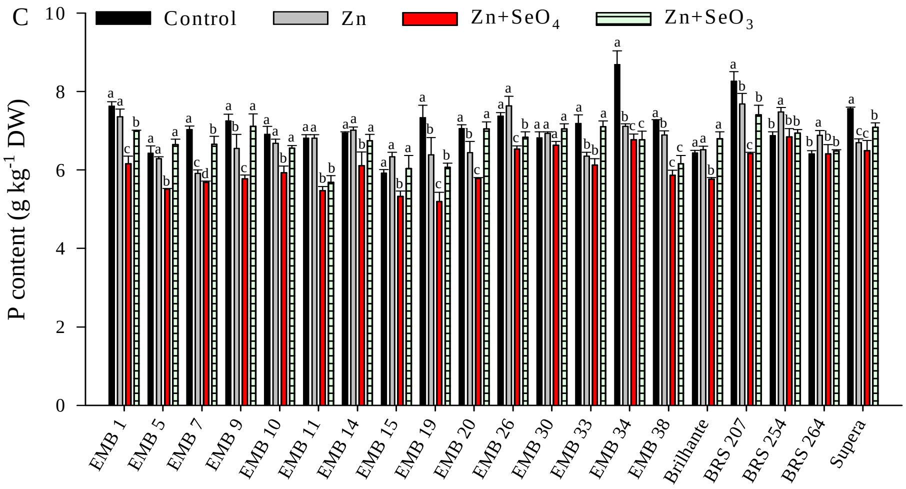


Figure 2. Effects of treatments on Zn (A), Se (B), P (C) and S (D) content in wheat shoots (flag leaves). Values shown are mean ± SE ($n = 3$). Different letters above the column indicate significant difference at $p \leq 0.05$ by Skott-Knott test.

Fig. 3


A
Control
Zn
Zn+SeO4
Zn+SeO3
Zn content (mg kg-1 DW)
B
Se content (µg kg-1 DW)
EMB 1
EMB 5
EMB 7
EMB 9
EMB 10
EMB 11
EMB 14
EMB 15
EMB 19
EMB 20
EMB 26
EMB 30
EMB 33
EMB 34
EMB 38
Brilhante
BRS 207
BRS 254
BRS 264
Supera



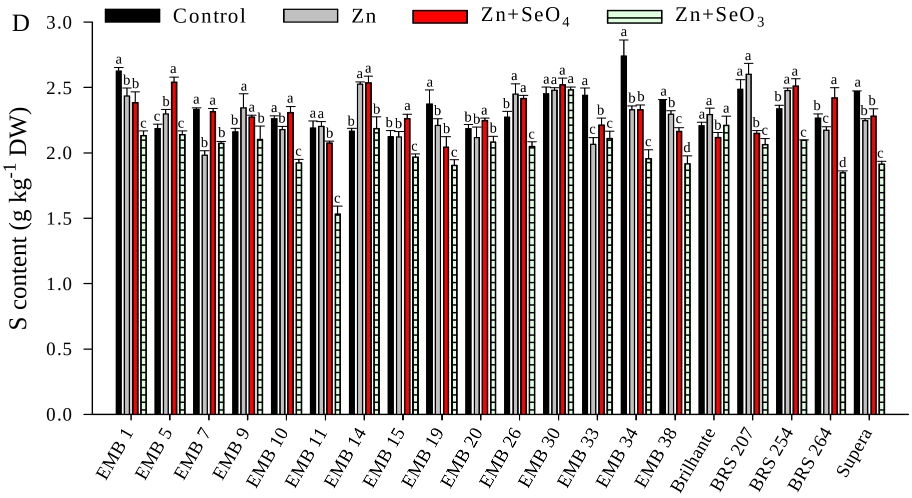
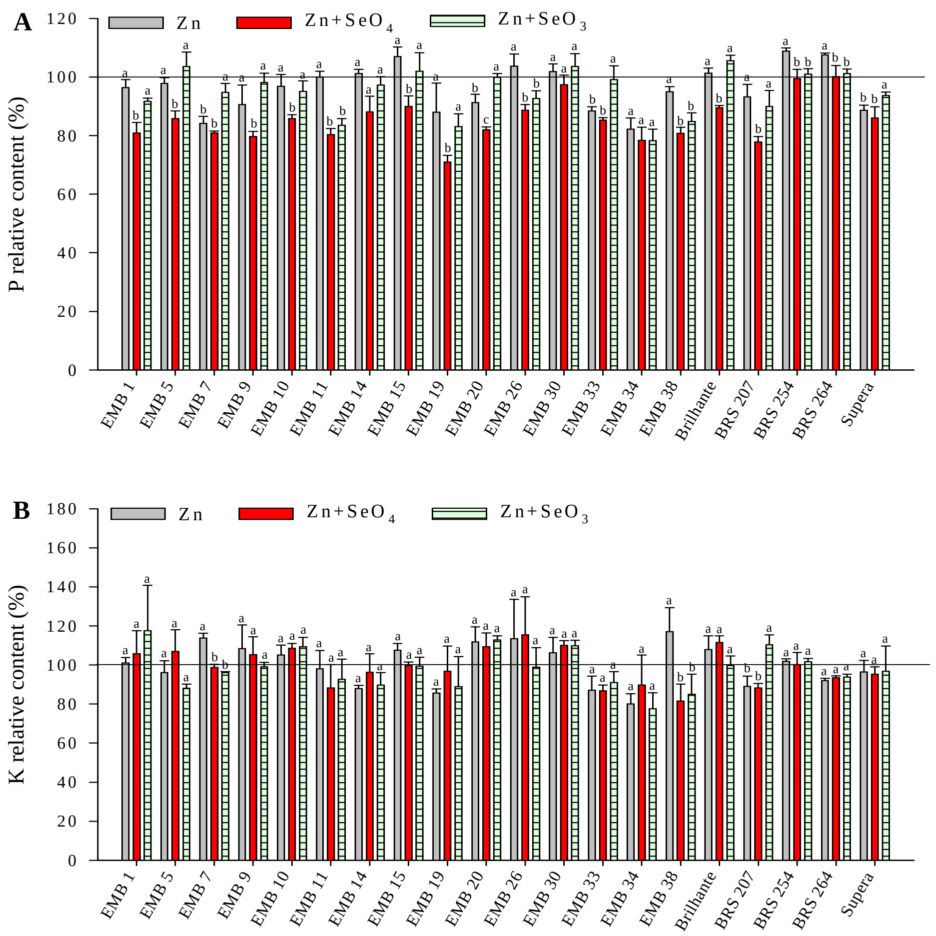
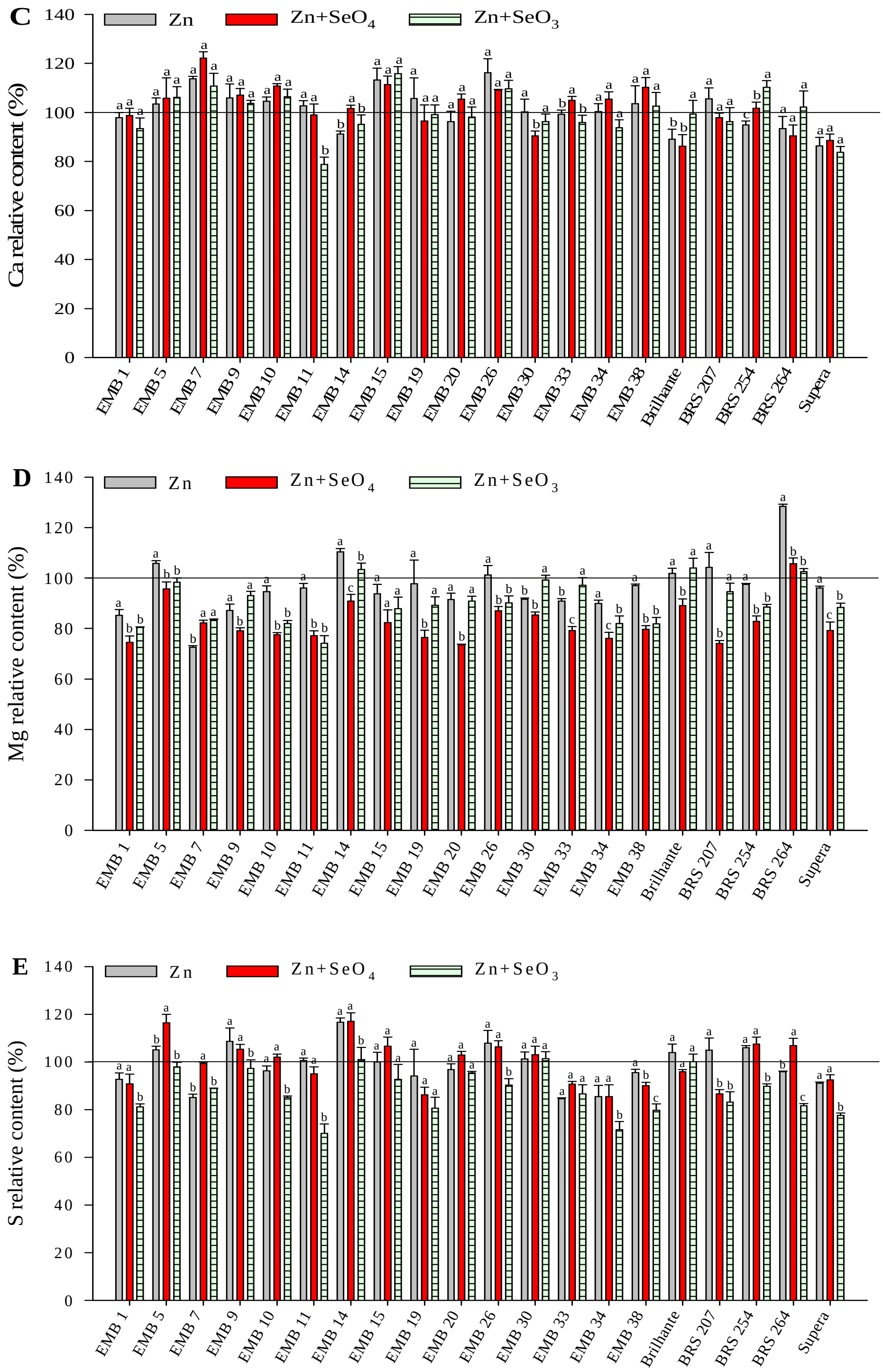
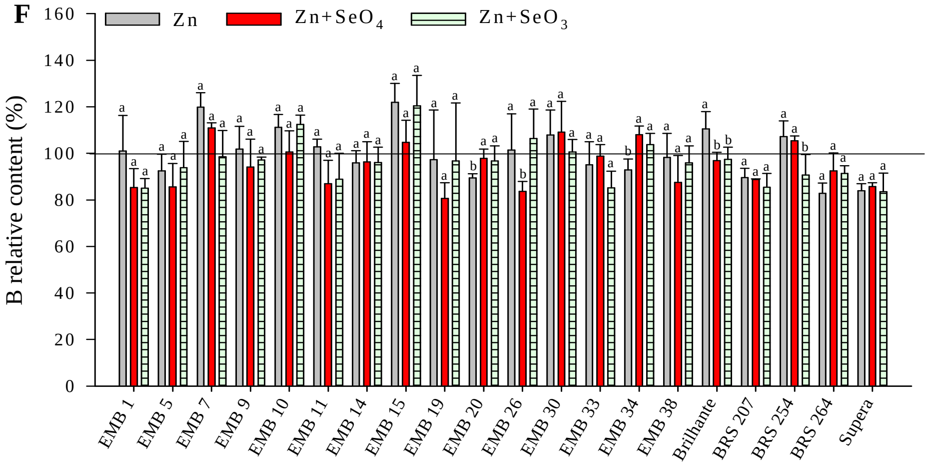


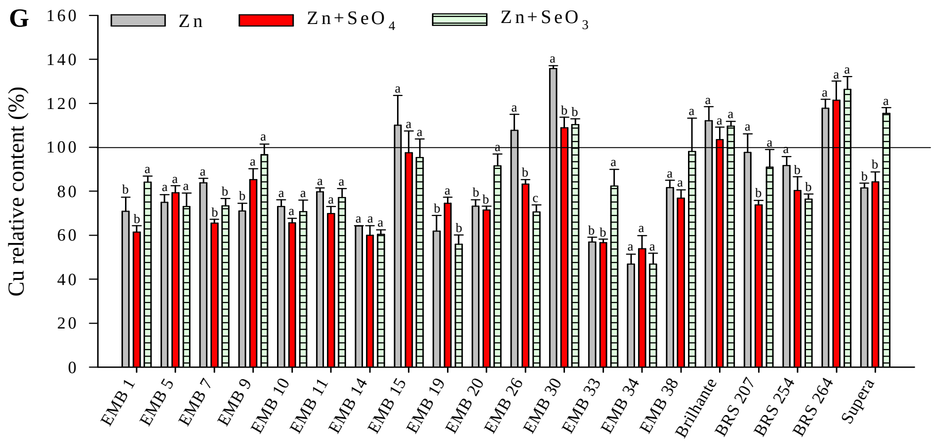
Figure 3. Effects of treatments on Zn (A), Se (B), P (C) and S (D) content in wheat grains. Values shown are mean ± SE (n = 3). Different letters above the column indicate significant difference at $p \leq 0.05$ by Skott-Knott test.

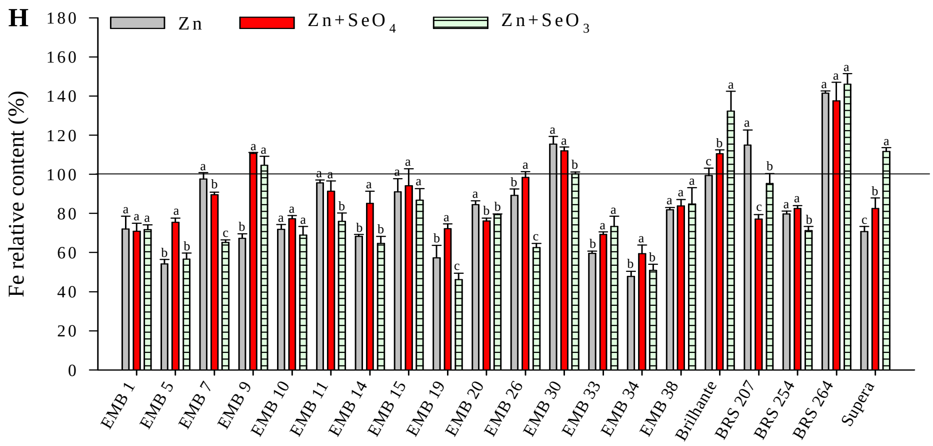
Supplemental figure S1.

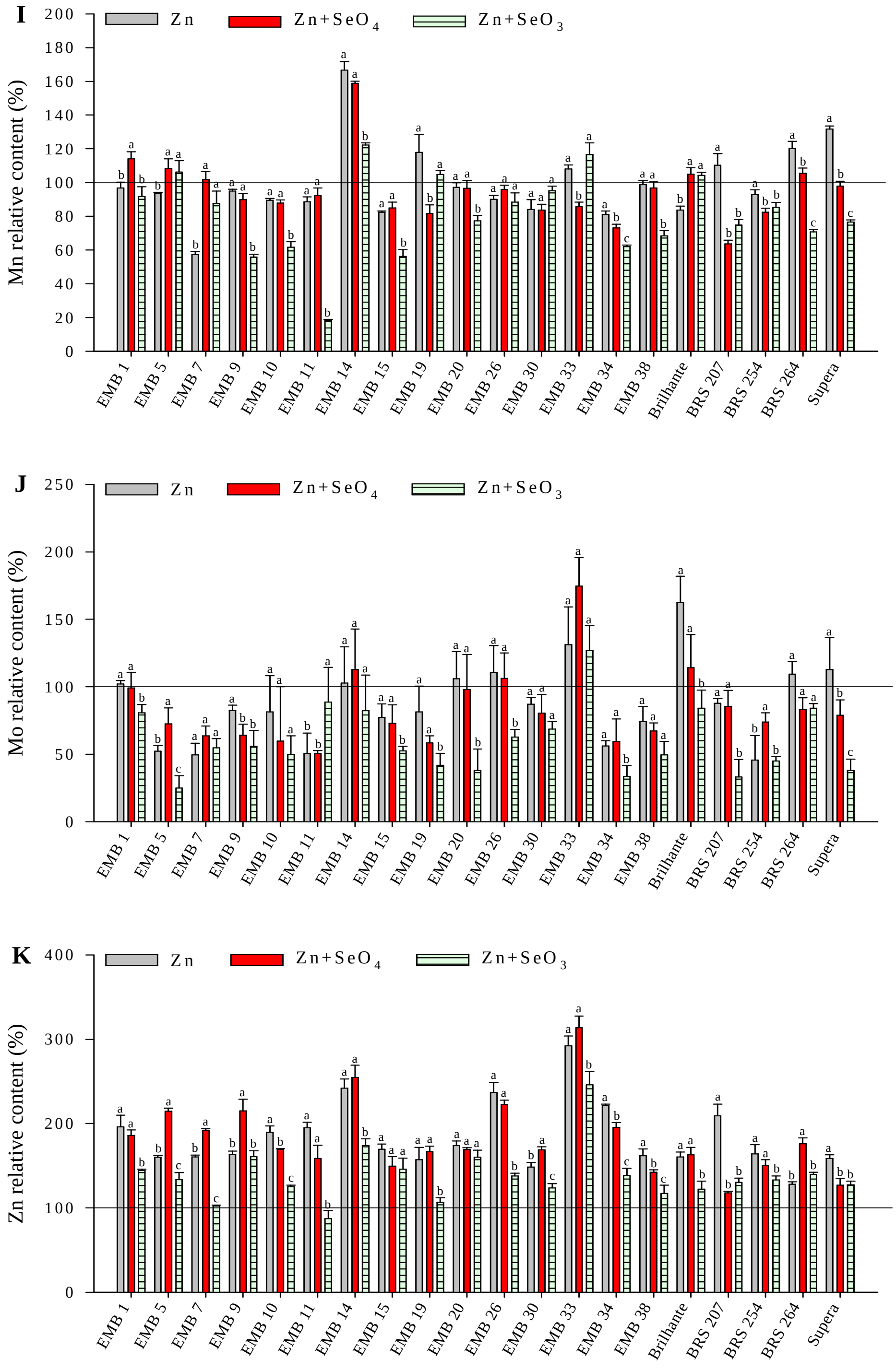



C
Zn
Zn+SeO4
Zn+SeO3
Ca relative content (%)
D
Mg relative content (%)
E
S relative content (%)
0
20
40
60
80
100
120
140
EMB 1
EMB 5
EMB 7
EMB 9
EMB 10
EMB 11
EMB 14
EMB 15
EMB 19
EMB 20
EMB 26
EMB 30
EMB 33
EMB 34
EMB 38
Brilhante
BRS 207
BRS 254
BRS 264
Supera


F
Zn
Zn+SeO4
Zn+SeO3
B relative content (%)
0
20
40
60
80
100
120
140
160
EMB 1
EMB 5
EMB 7
EMB 9
EMB 10
EMB 11
EMB 14
EMB 15
EMB 19
EMB 20
EMB 26
EMB 30
EMB 33
EMB 34
EMB 38
Brilhante
BRS 207
BRS 254
BRS 264
Supera


G
Zn
Zn+SeO4
Zn+SeO3
Cu relative content (%)
0
20
40
60
80
100
120
140
160
EMB 1
EMB 5
EMB 7
EMB 9
EMB 10
EMB 11
EMB 14
EMB 15
EMB 19
EMB 20
EMB 26
EMB 30
EMB 33
EMB 34
EMB 38
Brilhante
BRS 207
BRS 254
BRS 264
Supera


H
Zn
Zn+SeO4
Zn+SeO3
Fe relative content (%)
0
20
40
60
80
100
120
140
160
180
EMB 1
EMB 5
EMB 7
EMB 9
EMB 10
EMB 11
EMB 14
EMB 15
EMB 19
EMB 20
EMB 26
EMB 30
EMB 33
EMB 34
EMB 38
Brilhante
BRS 207
BRS 254
BRS 264
Supera



Supplemental Figure. Relative content of nutrients in wheat grains of 20 lines in response to Zn and Zn+Se treatments. The relative nutrient content were calculated by dividing the mineral contents following nutrient treatment with those in control solution. P (A), K (B), Ca (C), Mg (D), S (E), B (F), Cu (G), Fe (H), Mn (I), Mo (J) and Zn (K). Values shown are mean ± SE (n=3). Different letters above the column indicate significant difference at $p \leq 0.05$ for treatments.