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Effects of selenium (Se) application and arbuscular mycorrhizal (AMF) inoculation on soybean (*Glycine max*) and forage grass (*Urochloa decumbens*) development in oxisol

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Abstract

Fertilizer application can enhance the nutritional value of plants, such effects being influenced by the presence of arbuscular mycorrhizal fungi (AMF). Nutrients × AMF interactions are well-known for variety of elements but very little has been addressed on biofortification of selenium (Se) in plants grown in tropical soils. The purpose of this study was to evaluate the effect of Se application and AMF inoculation on growth and micronutrient contents on soybean plants as forage grass. The experiments were conducted in a completely randomized factorial design with five Se doses (0.0, 0.5, 1.0, 2.0 and 3.0 mg kg^{-1} for soybean plants, and 0.0, 0.5, 1.0, 3.0 and 6.0 mg kg^{-1} for forage plants), with and without AMF inoculation in three replicates. The results showed that soil Se had only slight effect on soybean growth but it caused a two-fold increase on grain yield. However, the growth of forage grass was enhanced by Se application when AMF was present. The AMF inoculation reduced benefit for soybean growth and yield but marked positive effect on forage grass at high doses of Se. Selenium contents in both plants were increased by its application in soil, being such effect proportional to soil applied doses. Selenium application and AMF inoculation had marked effects on micronutrients contents in both soybean plants and forage grass and they may contribute to Se and micronutrient biofortification.

Keywords: Biofortification; nutrient uptake; grasses; plant nutrition.
Abbreviations: Se_selenium; AMF_arbuscular mycorrhizal fungi.

Introduction

Selenium (Se) is an essential trace element for human and animal nutrition, and ingestion of Se enriched food and feed has relevant health benefits (Rayman 2012). One way to increase Se nutrition is through biofortification of foods, and the fact that the role of Se in plant growth is not clearly defined (Germ et al., 2007; Reis et al., 2018), and it represent a challenge for Se enrichment of plant material. Certain lower plants, such as algae, require Se for normal growth and reproduction, but yet this element is not known to be essential for the growth and development of higher plants (Natasha et al., 2018).

Se deficiency in human and animals occurs in many places around the world, and this seems to be due to low Se content in soils. The agricultural intervention that enhances the Se levels in edible parts of crop plants has been defined as biofortification (Garg et al., 2018), and this can be used as fortification and as supplementary strategy to enhance human and animal nutrition (Reis et al., 2009; Ebert and Jacob, 2007). There are many studies focused on the Se biofortification in different crops (Ramos et al., 2012; Ekanayake et al., 2015; Malagoni et al., 2015; Reis et al., 2018), but few of them has been carried out to investigate the influence of arbuscular mycorrhizal fungi (AMF) on the uptake of this element by plants (Durán et al., 2013; Goicoechea et al., 2015). This aspect has been over looked in tropical soils and crops, where AMF has strong effect on plant growth and nutrition. The AMF colonize the roots of about 80% of all know plant species establishing a mutual relationship to plant host and the associated fungus (Sosa-Hernández et al., 2018). In addition, to enhance plant growth, this symbiosis can improve the nutritional quality of plants by increasing concentration of mineral nutrients in plant tissue and grains (Lehmann et al., 2014). However, findings concerning these effects on Se are not consistent and they are usually dependent on the plant species and soil conditions. The presence of AMF can reduce the Se accumulation in plants (Munier-Lamy et al., 2007; Yu et al., 2011), increase the Se content (Larsen et al., 2006) or even has no effects (Patharajan and Raaman, 2012). Considering the high interest in Se biofortification in edible plants and the potential effect of AMF on nutrient uptake by plants, this study was developed to evaluate the effect of AMF inoculation and Se application on plant growth, Se and micronutrients contents in plants of soybean and forage grass.

Results and Discussion

Effect of Se on mycorrhizal colonization

In the present study, no mycorrhizal colonization was observed in roots of uninoculated plants. For both plants, the roots of inoculated plants were extensively colonized (average of 77 and 76% for soybean and grass, respectively), and were not affected be Se application.

Effect of Se and AMF on growth of soybean and brachiaria grass

Depending on the Se doses and AMF inoculation, different responses of the soybean plants and brachiaria grass in terms of growth were observed ($P < 0.05$; Fig. 1 and 2). It was observed only slight effects on growth of soybean plants, but Se application increase its uptake by roots, translocation to shoots and grains of soybean. At high concentration, Se inhibits plant growth given this element is chemically similar to sulfur and shares sulfur uptake and assimilation pathways. Beneficial effect of Se on lentil yield has been reported by Ekanayake et al. (2015), and this effect was showed mainly at low Se doses. As knew, the selenate assimilated by the plants uses the same enzymes involved in sulfate reduction, which are amino acid precursors and consequently of proteins, mainly in legumes plants that have high protein content, in that case its suppose the increases of grain yield is attributed to the great sodium assimilation by soybean plants (Thavarajah et al., 2015; Sors et al., 2005). The effects of AMF were quite evident and significant in shoot dry weight, but negligible for the others variables of soybean plants.

In brachiaria grass, there was a clear effect of AMF inoculation, especially at higher doses of Se. In presence of AMF plant growth response linearly to increasing doses of Se, whereas in the absence of this factor there was only a slight effect on dry weight of both roots and shoots (Fig. 2). Shoot dry weight of non-AMF plants was reduced at high Se doses. In other study with the same grass species, but without AMF inoculation, Ramos et al. (2012) showed that at 6 mg kg^{-1} of Se applied to soil the shoot yield was reduced by more than 20% compared to the absence of application.

Effects of Se and AMF on Se concentration in plants of soybean and brachiaria grass

Biofortification studies with Se and AMF are rare in fact. Our results showed, that at higher Se doses, the presence of AMF tended to favor the higher Se content in soybean plants (Fig. 1), while for forage grass the opposite was observed, especially at 6 mg kg^{-1} of Se (Fig. 2) ($P < 0.05$ of significance). Increase of Se concentration in soybean parts including grains were related to the doses applied up to 3.0 mg kg^{-1}, with positive effects on grains yields were marked with the first doses of Se (0.5 mg kg^{-1}), meaning an enhanced of almost a hundred percent on grain production, but no additional effects in higher doses, in contrast to what was observed for Se uptake and accumulation. The Se content in grains was increased by more than two-fold, as compared to non-Se treatment, reaching 0.25 mg kg^{-1}. For brachiaria grass, Se concentration in roots and shoots responded positively with increasing doses of Se and was much higher in roots than in shoots. This indicates high capacity of this grass to uptake Se from soil, but a rather low capacity to translocate this element to shoots. AMF contributed significantly to increase Se in shoots only at doses of 1.0 mg kg^{-1}. Therefore, the presence of AMF in the soil can enhances the nutrition value of brachiaria grass, when this element is supplied to soil at adequate level. Yu et al. (2011) observed that Se application with AMF inoculation in Chinese soil promoted reduction of Se in roots and shoots of alfalfa, maize, and soybean. Munier-Lamy et al. (2007) who studied the effect of mycorrhizal colonization on Se transfer and mobility in soil-plant system in a French soil, also found that mycorrhizal inoculation reduced the Se content in ryegrass.

In the present study, we used two important crop species, soybean has hundreds of uses from industrial products like crayons to food products and animal feeds (Johnson and Myers, 1995), while forage grass is an important source of livestock feed and contribute up to hundred percent of livestock daily diets in many countries. Brachiaria is one of the most important tropical forages supporting millions of livestock around the world (Boval and Dixon, 2012). Arthur (1972) reported that grains, cereals and forages are not reliable sources of Se since the amount found in such foods is highly dependent upon its content in most soils where the plants were grown. The Se content of a variety of soybean-based foods and forage grasses are below 0.1 mg kg^{-1} (Ferretti and Levander, 1976; Lebdosoekojo et al., 1980; Cuesta et al., 1993). Cardoso et al. (1997) who studied the nutritional content of twenty-four samples of brachiaria grass, found that grass Se concentrations were, respectively, 0.14 and 0.15 mg kg^{-1} for dry and wet seasons in the Amazon region, Brazil. Se requirement of most of the animals studied fall in the range of 0.05 to 1.25 mg kg^{-1} in the dry feed (Hafla, 2015). In the present study, we report the Se concentration and AMF inoculation enhance Se concentration in soybean and brachiaria grass, and such contents were in the range of adequate for soybean, but reached an excess in forage grass at doses higher 1.0 mg kg^{-1}.

Effect of Se and AMF on micronutrient levels

Contents of other micronutrients in soybean grains and grass shoots were generally not much affected by Se application, but were affected in a marked way by AMF inoculation $P < 0.05$ of significance (Fig. 3). For all nutrients studied (Cu, Fe, Mn, and Zn) AMF enhanced their contents in both grain and forage, therefore indicating the benefits of AMF symbiosis to food and feed nutritional quality. Such effects of AMF have been widely reported, especially for those nutrients with reduced mobility in soil (Faber et al., 1990; Kothari et al., 1990). Mycorrhizal plants could take up more metal nutrients via extraradical hyphae, which provide larger surface areas than the roots alone and reduce the distance for diffusion, thereby enhancing the absorption of immobile metal nutrients (Jakobsen et al., 1992). Previous studies without AMF inoculation found a decrease level of

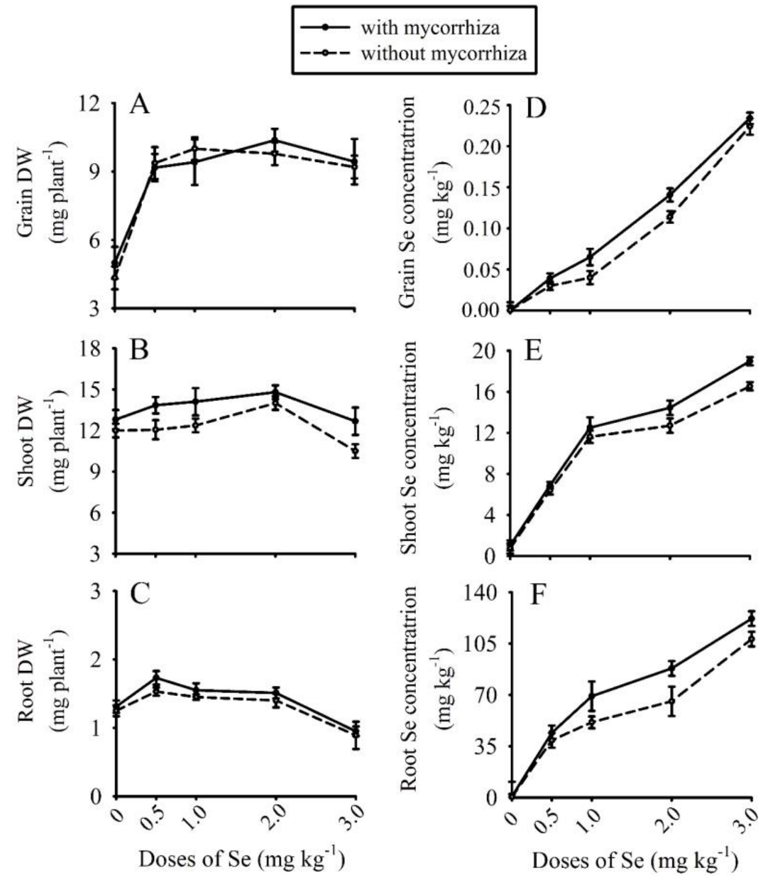

Fig 1. Grain (A), shoot (B) and root (C) dry matter yield, and concentration of Se in grain (D), shoot (E) and root (F) in soybean plants treated with doses of Se, and with and without mycorrhiza application.

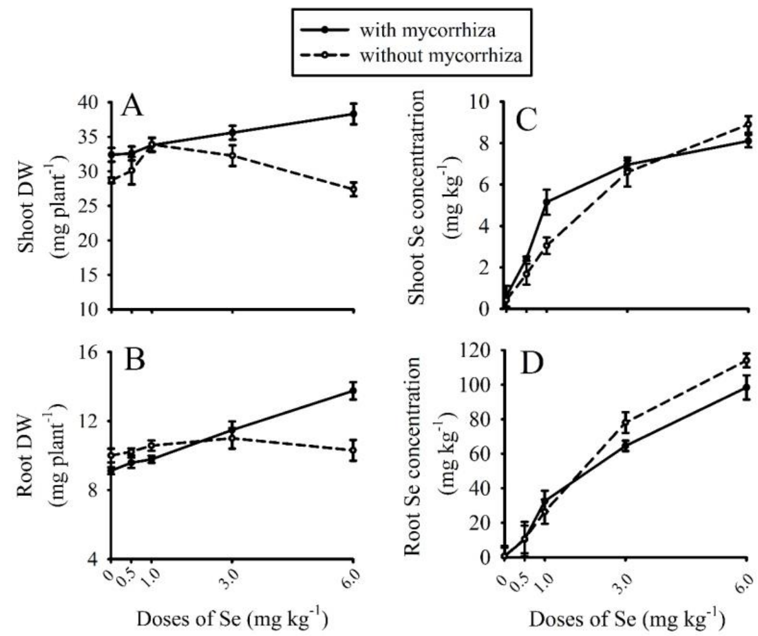

Fig 2. Shoot (A) and root (B) dry matter yield, and concentration of Se in shoot (C) and root (D) in grasses plants treated with doses of Se, and with and without mycorrhiza application.

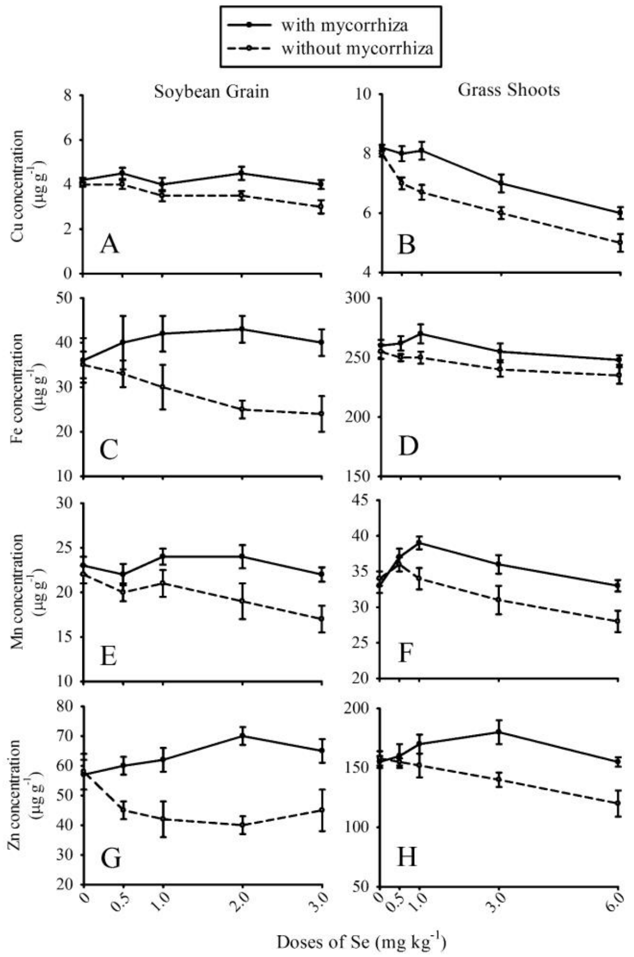

Fig 3. Concentrations of Cu, Fe, Mn, and Zn in soybean grain (A, C, E, G) and in grass shoots (B, D, F, H) treated with doses of Se, and with and without mycorrhiza application.

Micronutrients, when Se was applied in rice and forage grass (Boldrin et al., 2012; Ramos et al., 2012).

Materials and Methods

Experimental design and plant cultivation

The experiments were carried out in greenhouse at the Soil Science Department of the Federal University of Lavras, Brazil. Soil samples were taken from the 0-20 cm layer of an Oxisol with low Se level from the 'Cerrado' region, since it's the most abundant Brazilian soil. After air drying, the soil was sieved through a 2mm mesh to perform physical and chemical analyses as stated by Embrapa (1997). The chemical characteristics and physical results were: pH (water) = 4.9; H+Al (SMP extractor) = 3.20 cmolc dm^{-3}; Al (KCl extractor, 1 mol dm^{-3}) = 0.6 cmolc dm^{-3}; Ca (KCl extractor, 1 mol dm^{-3}) = 0.1 cmolc dm^{-3}; Mg (KCl extractor, 1 mol dm^{-3}) = 0.1 cmolc dm^{-3}; K (Mehlich 1) = 48.0 mg dm^{-3}; Na (Mehlich 1) = 0.0 mg dm^{-3}; P (Mehlich 1) = 0.8 mg dm^{-3}; OM = 1.40 g dm^{-3}; sand = 73 g dm^{-3}; silt = 2.0 g dm^{-3} and clay = 25.0 g dm^{-3}, Se= 0.06 mg dm^{-3}.
Two experiments were carried out using two crops, being soybean (*Glycine max (L.) Merr.)* and brachiaria grass *(Urochloa decumbens)*. The experimental design was randomized in a 5 × 2 × 3 factorial scheme, for both soybean and forage grass. The treatments consisted of five doses of Se, with and without AMF inoculation, and three replications. The Se doses for soybean experiment were 0.0; 0.5; 1.0; 2.0 and 3.0 mg kg^{-1}, while for forage grass were applied 0.0; 0.5; 1.0; 3.0 and 6.0 mg kg^{-1}, as previously studied by Yu et al. (2011) and Ramos et al. (2012), respectively. Sodium selenate purchased from Sigma-Aldrich-Saint Louis-USA was used in this study and applied into the soil as a solution, before sowing. The soils were sterilized in autoclave for two consecutive days at 120° C for one hour. After autoclaving, the soil was stored for 15 days for chemical stabilization, and then placed in pots of 5 kg capacity. In order to increase base saturation to 50%, all soils were incubated with limestone for 30 days. Before sowing the crops, pots with AMF treatment were inoculated with *Acaulospora morrowiae* (UFLA 469) and *Claroideoglomus etunicatum* (UFLA 217), these AMFs genre were chosen due its efficiency of colonization with both plants species (Bressan et al., 2001; Silva et al., 2006). The inoculated treatments received 50 dm^3 of soil-inoculum placed under the seeds at the sowing time, supplying about 1000 spores in each pot. The non-inoculated soil received 50 dm^3 of filtrated soil inoculum without mycorrhizal propagules. Each

crop received macronutrient fertilizer containing 100 mg N, 50 mg P, 100 mg K, and 40 mg S per kg of soil. Micronutrient application consisted of 3.6 mg Mn, 1.5 mg Cu, 5 mg Zn, 0.8 mg B and 0.15 mg Mo per kg of soil. In addition, for covering fertilizer of crops, 150 mg N and K per kg of soil was applied, splitted into three applications. While the experiment was being conducted, daily weighing of the pot-soil-plant set, replacing the lost volume with distilled water, rigorously controlled the soil moisture.

Analytical procedures

After 90 and 60 days of Se treatment, the soybean and forage grass were harvested, respectively. One gram of fresh roots for both plants was taken, clarified and colored with methyl blue (0.05%) (Koske and Gemma, 1989) to assess the mycorrhizal colonization through gridline intersect method (Giovannetti and Mosse, 1980). Shoots, roots and grains were washed in distilled water, placed in a paper bag and oven dried at 60º C until constant weight.

Concentration of Se and micronutrients in the plant materials were determined in a PerkinElmer Analyst 800 atomic absorption spectrophotometer (PerkinElmer Inc., San Jose, USA) with electrothermal atomization by (pyrolytic) graphite furnace essentially as described previously (Ramos et al., 2010). Briefly, dried tissues (approximately 500 mg) were weighed and acid digested in 10 mL HNO_3 in Teflon PTFE flasks (Corporation, Matthews, USA) and submitted to 0.76 MPa for 10 min in a microwave oven (CEM, model Mars 5 CEM Corporation, Matthews, USA). After cooling to room temperature, the extract was filtered (Whatman No. 40 filter) and diluted by adding 5 mL of bi-distilled water. Certified reference material (tomato leaves, NIST 1573a, National Institute of Standards and Technology (NIST), Gaithersburg, USA) were included for quality control. Blank and certified reference samples were analyzed along with every batch of digestion.

Statistical analysis

Normality test was applied to verify if the data had a normal distribution. All results showed normal distribution. Then, data was submitted to analysis of variance (ANOVA) followed by regression analyses at a 0.05 significance level of probability.

Conclusion

Soil-applied Se had only slight effect on soybean growth, but it caused a twofold increase in grain yield, whereas in forage grass growth was enhanced by Se application when AMF propagules were present in the soil.

The AMF inoculation had reduced benefit for soybean growth and yield, but marked positive effect on brachiaria grass at high doses of Se.

Selenium contents in both plants were increased by its application in soil, being such effect proportional to soil applied doses. Selenium application and AMF inoculation are found to increase levels of Cu, Fe, Mn, and Zn in both soybean plants and forage grass, and they may contribute to Se and micronutrient biofortification.

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Conflict of interest

The authors declare that they have no conflicts of interest.

References

Arthur D (1972) Selenium content of Canadian foods. Can Inst Food Sci Technol J. 5:165-169.

Bressan W, Siqueira JO, Vasconcellos CA, Purcino AAC (2001) Fungos micorrízicos e fósforo, no crescimento, nos teores de nutrientes e na produção do sorgo e soja consorciados. Pesq Agrop Bras. 36:315-323.

Boldrin PF, Faquin V, Ramos SJ, Guilherme LRG, Bastos CEA, Carvalho GS, Costa ETS (2012) Selenato e selenito na produção e biofortificação agronômica com selênio em arroz. Pesq Agrop Bras. 47:831-837.

Boval M, Dixon R (2012) The importance of grasslands for animal production and other functions: A review on management and methodological progress in the tropics. Animal. 6:748-762.

Cardoso EC, Vale WG, McDowell LR, Wikinson NS, Simão Neto M, Veiga JB, Lourenço Junior JB (1997) Seasonal variation of selenium, crude protein, and in vitro organic matter digestibility of *Brachiaria humidicola* from Marajó Island, Brasil. Commun Soil Sci Plant Anal. 28:1683-1691.

Cuesta PA, LR McDowell, WE Kunkle, F Bullock, A Drew, N Wilkinson, FG Martin (1993) Seasonal variation of soil and forage mineral concentration in north Florida. Commun Soil Sci Plant Anal. 24:335-347.

Durán P, Acuña JJ, Jorquera MA, Azcón R, Borie F, Cornejo P, Mora ML (2013) Enhanced selenium content in wheat grain by co-inoculation of selenobacteria and arbuscular mycorrhizal fungi: A preliminary study as a potential Se biofortification strategy. J Cer Sci. 57:275-280.

Ebert R, Jakob F (2007) Selenium deficiency as a putative risk factor for osteoporosis. Int Congr Ser. 1297:158-164.

Ekanayake LJ, Thavarajah D, Vial E, Shatz B, McGee R, Thavarajah P (2015) Selenium fertilization on lentil (*Lens culinaris* Medikus) grain yield, seed selenium concentration, and antioxidant activity. Field Crops Res. 177:9-14.

Embrapa (1997) Manual of methods for soil analysis. Embrapa, Rio de Janeiro.

Faber BA, Zasoski RJ, Burau RG, Uriu K (1990) Zinc uptake by corn affected by vesicular-arbuscular mycorrhizae. Plant Soil. 129:121-131.

Ferretti R, Levander OA (1976) Selenium content of soybean foods. J Agric Food Chem. 24:54-56.

Garg M, Sharma N, Sharma S, Kapoor P, Kumar A, Chunduri V, Arora (2018) Biofortified crops generated by breeding, agronomy, and transgenic approaches are improving lives of millions of people around the world. Front Nutr. 5:12.

Germ M, Stibilj V, Kreft I (2007) Metabolic importance of selenium for plants. Eur J Plant Sci Biotechnol. 1:91-97.

Giovannetti M, Mosse B (1980) An evaluation of techniques for measuring vesicular arbuscular mycorrizal infection in roots. New Phyt. 84:489-500.

Goicoechea N, Garmendia I, Fabbrin EG, Bettoni MM, Palop JA, Sanmartín C (2015) Selenium fertilization and mycorrhizal technology may interfere in enhancing bioactive compounds in edible tissues of lettuces. Sci Hort. 195:163-172.

Hafla A. The impostance of micro-minerals: Selenium (2015) Agri-King's Newsflash Publication.

Jakobsen I, Abbott LK, Robson AD (1992) External hyphae of vesicular-arbuscular mycorrhizal fungi associated with *Trifolium subterraneum* L. 1. Spread of hyphae and phosphorus inflow into roots. New Phytol. 120:371-380.

Johnson L, Myers D (1995) Industrial uses for soybeans. In: Erickson DR (ed) Practical handbook of soybean processing and utilization. 1st edn. AOCS Press, Illinois. 380-427.

Koske R, Gemma J (1989) A modified procedure for staining roots to detect VA mycorrhizas. Myc Res. 92:486-488.

Kothari SK, Marschner H, Römheld V (1990) Direct and indirect effects of VA mycorrhizal fungi and rhizosphere microorganisms on acquisition of mineral nutrients by maize (*Zea mays* L.) in a calcareous soil. New Phytol. 116:637-645.

Larsen EH, Lobinski R, Burger-Meyer K, Hansen M, Ruzik R, Mazurowska L, Rasmussen, PH, Sloth JJ, Scholten O, Kik C (2006) Uptake and speciation of selenium in garlic cultivated in soil amended with symbiotic fungi (mycorrhiza) and selenate. Anal Bioanal Chem. 385:1098-1108.

Lebdosoekojo S, CB Ammerman, NS Raun, J Gomes, RC Littell (1980) Mineral nutrition of beef cattle grazing native pastures on the eastern plains of Colombia. J Anim Sci. 51:1249-1260.

Lehmann A, Veresoglou SD, Leifheit EF, Rillig MC (2014) Arbuscular mycorrhizal influence on zinc nutrition in crop plants – A meta-analysis. Soil Biol Biochem. 69:123-131.

Malagoli M, Shiavon M, dall'Acqua S, Pilon-Smits EAH (2015) Effects of selenium biofortification on crop nutritional quality. Front Plant Sci. 6:1-5.

Munier-Lamy C, Deneux-Mustin S, Mustin C, Merlet D, Berthelin J, Leyval C (2007) Selenium bioavailability and uptake as affected by four different plants in a loamy clay soil with particular attention to mycorrhizae inoculated ryegrass. J Environ Radioact. 97:148-158.

Natasha, Shahid M, Naizi NK, Khalid S, Murtaza B, Bibi I, Rashid MI (2018) A critical review of selenium biogeochemical behavior in soil-plant system with an influence to human health. Environ Pollut. 234:915-934.

Patharajan S, Raaman N (2012) Influence of arbuscular mycorrhizal fungi on growth and selenium uptake by garlic plants. Arch Phytopath Plant Protect. 45:138-151.

Ramos SJ, Faquin V, Guilherme LRG, Castro EM, Ávila FW, Carvalho GS, Bastos CEA, Oliveira C (2010) Selenium biofortification and antioxidant activity in lettuce plants feed with selenate and selenite. Plant Soil Environ. 56:584-588.

Ramos SJ, Ávila FW, Boldrin PF, Pereira FJ, Castro EM, Faquin V, Reis AR, Guilherme LRG (2012) Response of brachiaria grass to selenium forms applied in a tropical soil. Plant Soil Environ. 58:521-527.

Rayman MP (2012) Selenium and human health. Lancet. 379:1256-1268.

Reis LSLS, Chiacchio SB, Pardo RT, Couto R, Oba E, Kronka SN (2009) Effect of the supplementation with selenium on serum concentration of creatine kinase in cattle. Arch Zoot. 58:753-756.

Reis HPG, Barcelos JPQ, Furlani Junior E, Santos EF, Silva VM, Moraes MF, Putti FF, Reis AR (2018) Agronomic biofortification of upland rice with selenium and nitrogen and its relation to grain quality. J Cereal Sci. 79:508-515.

Silva S, Siqueira JO, Soares CSFR (2006) Fungos micorrízicos no crescimento e na extração de metais pesados pela braquiária em solo contaminado. Pesq Agrop Bras. 41:1749-1757.

Sors TG, Ellis DR, Salt DE (2005) Selenium uptake, translocation, assimilation and metabolic fate in plants. Photosynth Res. 86:373-389.

Sosa-Hernández M, Roy J, Hempel S, Kautz T, Kopke U, Uksa M, Schloter M, Caruso T, Rillig MC. Subsoil arbuscular mycorrhizal fungal communities in arable soil differ from those in topsoil. Soil Biol Biochem. 117:83-86.

Thavarajah D, Thavarajah P, Ekanayake LJ, Vial E, Gebhardt M, Lacher C, Kumar S, Combs GF (2015) Will selenium increase lentil (*Lens culinaris* Medik) yield and seed quality? Front Plant Sci. 6:1-9.

Yu Y, Zhang S, Wen B, Huang H, Luo L (2011) Accumulation and speciation of selenium in plants as affected by arbuscular mycorrhizal fungus *Glomus mosseae*. Biol Trace Elem Res. 143:1789-1798.