Application of sodium selenate to cowpea (*Vigna unguiculata* L.) increases shoot and grain Se partitioning with strong genotypic interactions

Short Title: Genotypic variation of cowpea under Selenium application

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Abstract

Background: Cowpea is a crop widely used in developing countries due its rusticity. Besides its rich genotypic variability, most breeding programs do not explore its potential to improve elements uptake. Selenium (Se) is a scarce element in most soils, resulting in its deficiency being common in human diets. This study aimed to evaluate the interaction between biofortification with Se and genotypic variation in cowpea, on the concentrations of Se in roots, leaves + stem and grains.

Methods: Twenty-nine cowpea genotypes were grown in a greenhouse in the absence (control) and presence of Se (12.5 µg Se kg^{-1} soil) as sodium selenate, in fully randomized scheme. The plants were cultivated until grains harvest. The following variables were determined: roots dry weight (g), leaves + stems dry weight (g), grains dry weight (g), Se concentration (mg kg^{-1}) in roots, leaves + stems and grains, and Se partitioning to shoots and grains.

Results: Selenium application increased the Se concentration in roots, leaves + stems and grains in all genotypes. At least twofold variation in grain Se concentration was observed among genotypes. Selenium application did not impair biomass accumulation, including grain dry weight. Genotype "BRS Guariba" had the largest Se concentration in grains and leaves + stems. Genotype MNC04-795F-158 had the largest partitioning of Se to shoots and grain, due to elevated dry weights of leaves + stems and grain, and high Se concentrations in these tissues.

Conclusion: This information might be valuable in future breeding programs to select for genotypes with better abilities to accumulate Se in grain to reduce widespread human Se undernutrition.

Keywords: *Vigna unguiculata* (L.) Walp; sodium selenite; selenium partitioning; grain quality; biofortification; pulses

1. Introduction

Currently, about 820 million people worldwide are estimated to consume insufficient food to meet their dietary energy requirements [1]. Further, more than half of the world's population are likely to consume insufficient quantities of one or more micronutrients [2]. This is, in part, a consequence of plant breeding aimed at increasing crop yield, which can reduce tissue nutrient concentrations through growth dilution [3]. Crop-breeding programs need to expand their focus from yield alone, to also increasing nutrient concentrations in edible plant tissue to combat human micronutrient malnutrition.

Selenium (Se) is an essential element for human and animal nutrition, serving in antioxidant processes, and contributing to proper immune system and thyroid functioning [4]. It is also considered a chemo-preventative agent, since appropriate Se nutrition is able to reduce the risk of cancer, and may delay or reduce the prevalence of its recurrence [5]. In plant nutrition, Se is considered a beneficial element, acting in the biotic and abiotic stress response as an antioxidant promoter [6,7, 8,9,10].

Despite its nutritional importance, Se is a trace-element, usually present in low concentrations in the soil [11] and in most foods. Plant-based diets often result in Se undernutrition in both humans and animals, leading to Se deficiency symptoms [12]. It is estimated that more than 1 billion people suffer from Se deficiency [13]. Among human micronutrient deficiency risks, Se ranks as the third most prevalent, with particularly high deficiency risks in Africa [14].

Cowpea (*Vigna unguiculata* (L.) Walp.) is a legume (Fabaceae) believed to have originated in West Africa [15]. It is rich in protein [16] and well adapted to low fertility soils, high temperatures and drought conditions [17]. These traits make cowpea a good food source to complement other edible plants, and it is currently used to provide protein to diets in African, Asian and Latin American countries [16,18]. The use of improved cowpea genotypes with higher yields, might reduce poverty and lead to increase household income [19]. An additional benefit of cowpea in the human diet is the potential for its sprouts to help reduce colorectal-cancer [20].

Breeding programs for cowpea aim to develop improved lines with high grain yield potential, resistance to biotic stresses, tolerance to abiotic factors, adaptation to major production, and traits preferred by consumers and producers [21]. Recently, breeders have focused on cowpea genotypes with the potential to accumulate micronutrients in edible tissue, aiming to benefit human health by combating undernutrition [22].

There is considerable genotypic variation in seed Se concentration in many grain crops [16]. In rice, roots of cultivars that accumulate more Se appear to release more organic acids, which increases Se availability in rhizosphere [23]. However, some rice genotypes accumulate more Se in roots and leaves, with limited translocation of Se to grain [24], thus impacting on their effectiveness in improving human Se nutrition. These studies exemplify the importance of selecting genotypes that not only acquire Se effectively but also translocate Se to edible portions, which are independent processes.

The effect of Se applications on seed Se concentration in crops is well-established [25]. For example, in rice, a Se application rate of 25 g ha^{-1} increased grain Se concentration from 0.03 to 0.32 mg kg^{-1} and also increased albumin and glutelin in seeds [8]. In wheat, Se application at a rate of 10 g ha^{-1} resulted in substantial increases in the concentrations of Se and selenomethionine in both grain and bread [26] and Se application at a rate of 21 g ha^{-1} increased both Se concentration in grain and grain yield [27]. In cowpea, a Se application rate of 10 g ha^{-1} provided sufficient Se to increase daily human Se intake by up to 14 μg d^{-1}, based on current dietary trends, without impairing cowpea yield and biomass [28].

Since little is known about genotypic variation in Se accumulation in cowpea, this study aimed to evaluate variation in the ability of 29 cowpea genotypes to accumulate Se in roots, shoots and grains, as well as the interactions between genotypic effects and Se application rates.

2. Materials and methods

2.1.Experimental design

The experiment was conducted in a greenhouse at São Paulo State University (UNESP) in the municipality of Tupã, São Paulo State, Brazil. Twenty-nine cowpea genotypes (Table 1) were cultivated in the absence (control) and presence of additional Se (12.5 μg Se kg^{-1} soil). Cowpea genotypes were received from Brazilian Agricultural Research Corporation (EMBRAPA). The experiment had a completely randomized design, with three replicates per genotype per Se treatment, totaling 174 pots.

The soil was collected from the experimental farm at São Paulo State University and sieved to fill pots to the volume of 5 dm^3 in November 2016. Soil chemical characteristics were as follows: pH ($CaCl_2$ 0.01 M) 4.6; phosphorus (resin), sulfur (calcium phosphate), boron (hot water), copper (DTPA), iron (DTPA), manganese (DTPA) and zinc (DTPA): 6, 3, 0.07, 0.5, 11, 12 and 0.2 mg dm^{-3} respectively; potassium (resin), calcium (resin), magnesium (resin), H+Al (SMP buffer) and cation exchange capacity: 0.9, 5, 3, 16 and 24.9 mmolc dm^{-3} respectively; base saturation: 36%. These chemical characteristics were determined as described by Raij et al [29]. Total and exchangeable Se concentration in unamended soil were 45 μg kg^{-1} and 3.2 μg kg^{-1}, respectively. To determine soil Se concentrations, 4 g of air-dried soil was weighted and added into a 50 mL centrifuge tube, in which was added 20 mL of 0.01M KNO_3. A rotary shaker was used to homogenize the solution for 2 hours, then a centrifugation was performed for 30 min at 1650xg. After centrifugation, into a < 0.22 μm syringe filter 9 mL of the supernatant was pipetted. The supernatant was filtered into a tube containing 0.1 M KH_2PO_4 and 10% tetramethylammonium hydroxide (TMAH) for determination of total and exchangeable Se, respectively. The determination was carried out using an inductively coupled plasma-mass spectrometry (ICP-MS; Thermo Fisher Scientific iCAPQ, Thermo Fisher Scientific, Bremen, Germany), as described by Silva et al [28].

Prior to sowing cowpea seeds, each pot received 1.25 g of lime, 0.17 g KCl, and 0.46 g single superphosphate. The soil was incubated 30 days prior sowing the seeds. For inoculation, a peat inoculum specific for cowpea was used (2.0 x 10^9 colony forming units g^{-1}, strain SEMIA 6462, BIOMAX, São Joaquim da Barra city, Brazil), at 8 g kg^{-1} of seed. The inoculum was dissolved in a

sugar solution (1 mL of water per gram of inoculant, 10% sugar) and then added and mixed with the seeds. Sowing was performed on December 29 2016. Emergence began on January 5 2017, 7 days after sowing (DAS). An application of urea (0.10 g pot^{-1}), was made 27 DAS. The pots were irrigated by a custom computerized irrigation system. Each pot was irrigated five times daily for one minute each time, at a flow rate of 100 mL min^{-1} via a low-density polyethylene (LDPE) pipes. Data regarding temperature and potential evapotranspiration were presented in figure A.1.

2.2.Procedure and sampling

According to general recommendations for cowpea cultivation, 29 days after emergence (DAE), each pot received 20 mL of a solution containing urea (0.22 g pot^{-1}), KCl (0.17 g pot^{-1}), and single superphosphate (0.45 g pot^{-1}). The same fertiliser applications were made 36 DAE and 61 DAE. Selenium treatments were applied to the relevant pots 44 DAE at a rate of 12.5 µg kg^{-1} soil per pot, using sodium selenate. The application rate and salt were selected according to previous studies of the group regarding Se application rates and sources in cowpea [28].

Harvest was performed across a series of days for each genotype according to the pod's maturity. The material was separated into grains, leaves + stems and roots. Root material was washed with deionized water. Root, leaves + stems, and seeds of cowpea plants were dried in an oven at 40 °C for 72 hours to a constant mass to measure the dry weight (DW) of grains, leaves + stems and roots. After determining its dry weight, sample material was ground and homogenised in a Wiley mill for chemical analysis.

2.3.Digestion and nutritional analysis of roots, leaves + stems and grains

Subsamples (~0.20 g) of dried, milled root, leaves + stems and grain materials were weighed (exact weights recorded) and digested in digestion tubes of a perfluoroalkoxy (PFA) liner material containing 2 mL 70 % Trace Analysis Grade HNO_3, 1 mL Milli-Q water, and 1 mL H_2O_2. Prior to elemental analysis, the digestates were diluted 1-in-10 using milli-Q water. The digestion of plant

material and analysis by ICP-MS were performed according to the methods of Thomas et al [30]. Data processing was undertaken using Qtegra™ software (Thermo Fisher Scientific).

2.4.Selenium translocation estimative

Selenium partitioning was calculated for each genotype following Abichequer & Bohnen [31] using the following equations:

$$Se\ partitioning\ to\ shoot\ (\%) = \frac{(B \times E) + (C \times F)}{(A \times D) + (B \times E) + (C \times F)} \quad \text{(Equation 1)}$$

$$Se\ partitioning\ to\ grains\ (\%) = \frac{(C \times F)}{(A \times D) + (B \times E) + (C \times F)} \quad \text{(Equation 2)}$$

where:

A – Root dry weight (kg)

B – Leaves + stems dry weight (kg)

C – Grains dry weight (kg)

D – Root Se concentration (μg kg^{-1})

E – Leaves + stems Se concentration (μg kg^{-1})

F – Grains Se concentration (μg kg^{-1})

2.5.Statistical analysis

The normality of the data was determined using the Anderson-Darling normality tests; Leven's test was used to determine homogeneity, then a variance analysis (F test) was performed. Mean differences between treatments were compared using a Scott-Knott test at 5% probability. Analyses were performed using R (version 3.5.1; source: https://cran.r-project.org/bin/windows/base/old/). A decision matrix was performed for variables that presented

interaction between Se application and genotypes, to helps do pinpoint which is the best genotype for each characteristic.

3. Results

The effect of Se on the dry weights of roots, leaves + stems and grain of the 29 cowpea genotypes is reported in Fig. 1. Regardless of the Se application, genotypes 1, 2, 4, 5, 6, 7, 8, 13, 20, 21 and 29 had larger root dry weight than the other genotypes (Fig. 1a, Table A.2; $p \leq 0.05$). The leaf + stem dry weights and grain dry weights were not affected by Se application (Fig. 1b-c; Table A.1). However, regardless of Se application, genotypes 4, 6, 8, 12, 17, 19, 21, 23, 24, 25 and 26 had larger leaf + stem dry weights than other genotypes, and genotypes 4, 6, 10, 12, 15, 17, 19, 23, 24, 25, and 26 had larger grain dry weights than other genotypes (Fig. 1c; Table A.2).

An interaction was observed between Se application and genotype in root dry weight ($P<0.05$; Table A.1). Selenium application resulted in an increase in root dry weight in genotypes 8 and 19, and a decrease in root dry weight in genotype 4; root dry weight was not affected by Se application in the other genotypes (Fig. 1a, Table A.2).

The application of Se increased Se concentration in grain, leaves + stems and roots of most genotypes (Fig. 2, 3 and 4, respectively). However, an interaction between Se application and cowpea genotypes was observed for Se concentrations in grain, leaves + stems and roots ($P<0.0001$; Table A.1). There was no difference in Se concentration in any tissue among genotypes grown without Se application. However, wide genotypic variation was observed in Se concentrations of grain, leaves + stems and roots when Se was applied (Fig. 2, 3 and 4, respectively).

Selenium application increased grain Se concentration in all 29 genotypes (Fig. 2). The increase in grain Se concentration when Se was applied ranged from 549 µg Se kg^{-1} dry weight in genotype 16 to 2462 µg Se kg^{-1} dry weight in genotype 5 (Fig. 2; Table A.3). A significant interaction was observed between Se application and genotype on Se concentration in grain ($P<0.0001$; Table A.1).

An increase in Se concentration in leaves + stems in response to Se application was observed in most genotypes, except genotypes 8, 9, 21, 22 and 27 (Fig. 3). Genotype 19 showed the smallest increase in the Se concentration of leaves + stems (313 µg kg^{-1}) and genotype 5 the largest increase in the Se concentration of leaves + stems (1218 µg kg^{-1}) following Se application (Fig. 3; Table A.3). There was a significant interaction between Se application and genotype on the Se concentration in leaves + stems ($P<0.01$; Table A.1).

Selenium application did not affect root Se concentration in genotypes 1, 18, 21, 22 and 29, but increased the root Se concentration of the other 25 genotypes (Fig. 4; $p\leq0.05$). The largest root Se concentration after Se application was 1755 µg kg^{-1} dry weight in genotype 28 (Fig. 4; Table A.3).

Regarding Se concentrations in grains, leaves + stems and roots from Se-biofortified genotypes, five, four and three distinct groups were observed according to Scott-Knott test, respectively (Fig. 2, 3, 4, respectively, Table A.3). For grain Se concentration, genotype 5 had the largest mean Se concentration of 2462 µg kg^{-1} dry weight when Se was applied (Fig. 2; Table A.3). For leaves + stems, two genotypes, 5 and 12, had the largest mean Se concentrations, which were equal to or greater than 1183 µg kg^{-1} dry weight when Se was applied (Fig. 3; Table A.3). For roots, genotypes 13, 24 and 28, had the largest mean Se concentrations, which were equal or greater than 1444 µg kg^{-1} when Se was applied (Fig. 4; Tables A.3).

Selenium partitioning to the shoot was evaluated by comparing Se accumulation in leaves + stems (shoot) plus grains with Se accumulation in the whole plant. The partitioning of Se to the shoot was affected by genotype and by Se application ($P<0.0001$; Table A.1). There was also an interaction between Se application and genotype on the partitioning of Se to the shoot ($P<0.0001$; Table A.1). The application of Se influenced Se partitioning to the shoot in all genotypes except genotypes 9 and 22, according to the Scott-Knott test (Table A.4; $p\leq0.05$). Genotypes were divided into three groups according to the Scott-Knott test, for their partitioning of Se to the shoot (Fig. 5). For group a, Se partitioning to the shoot was between 64% (genotype 15) and 91% (genotype 5)

following Se application; for group b, Se partitioning to the shoot was between 47% (genotype 20) and 61% (genotype 18) following Se application; and for group 3, Se partitioning to the shoot ranged from 28% (genotype 9) to 37% (genotype 19) following Se application (Fig. 5, Table A.4). Considering all genotypes, the mean Se partitioning to shoot was 61.5% following Se application, but only 14.2% in the absence of Se application (Fig. 5, Table A.4).

Selenium partitioning to grains was also evaluated. The mean Se partitioning to grains was 20.0% when Se was applied to plants, but only 2.3% when no Se was applied (Table A.5). Genotypes were divided into two groups for their partitioning of Se to grain, regardless of whether Se was applied or not, according to the Scott-Knott test (Fig. 6, Table A.3). Group a was represented by genotypes in which Se partitioning was between 11.8%, (genotype 15) and 17.8% (genotype 26), and group b was represented by genotypes in which Se partitioning was between 5.3% (genotype 27) and 11.5% (genotype 3; Fig. 6, Table A.5). Although effects of Se application and genotype were observed for Se partitioning to grains, there was no interaction between these factors (Table A.1; Table A.5).

4. Discussion

Selenium application had little effect on plant growth in this study. Selenium application had no effect on leaf + stem and grain dry weights in any of the genotypes studied (Fig. 1b-c). However, the root dry weight of genotype 4 (BRS Cauamé) was reduced by Se application (Fig. 1a), suggesting a potential impairment in root development following Se application in some genetic backgrounds. Genotypes 8 (BRS Marataoa) and 19 (MNC01-631F-20-5) exhibited larger root dry weights following Se application (Fig. 1a).

The range of tissue Se concentrations is relatively narrow between those causing beneficial and toxic effects in plants, varying from 100 μg kg^{-1} for beneficial effects, to 1500 μg kg^{-1} to cause toxic effects [6,9,10]. Increased growth or yield of plants following Se application is occasionally observed [6,10] and, for example, the seedling biomass of nine out of 26 genotypes of lentil [32]

and the yield of wheat [27] have been increased by the application of Se. By contrast, application of excess Se impairs plant growth under field, glasshouse and laboratory conditions [9,10,33]. Thus, the observation that a substantial increase in Se concentrations in cowpea tissues can be achieved without a reduction in cowpea yield at the Se application rates used in this study is valuable agronomic information.

The Se concentrations in control plants was considered the typical Se concentration range for the cowpea genotypes used in this study. In grain from control plants, the typical Se concentration varied from 4.31 µg kg^{-1} in genotype 25 (Patativa) to 159.91 µg kg^{-1} in genotype 28 (Pingo de Ouro 2; Fig. 2). The reported Se concentrations in seeds and grain of crops grown without Se fertiliser applications varies widely [10]. For example, Thavarajah et al [32], studying Se concentration in grains of 191 lentil accessions grown without the application of Se fertiliser, observed that grain Se concentrations of most of the accessions was within the range of 250 µg kg^{-1} to 750 µg kg^{-1} of Se, although some accessions reached concentrations of more than 2000 µg kg^{-1} . However, no Se was detected in mature seeds of common bean genotypes when no Se fertiliser was applied [34]. The relatively low Se concentrations observed here in grain of cowpea observed in the present study (Fig. 4) might be due to the small amounts of Se acquired and the small percentage of the Se acquired being partitioned to grain (Fig. 6). In the control plants, Se partitioning to grain was only about 2% (Fig. 6, Table A.5) and most of the Se in plants grown without Se fertiliser application tended to be accumulated in shoots (leaves + steam) (Fig. 5, Table A.4). The typical Se concentration in leaves + stems varied from 8.42 µg kg^{-1} in genotype 23 (MNC04-792F-146) to 78.16 µg kg^{-1} in genotype 22 (MNC04-792F-143; Fig. 3). The typical Se concentration in roots varied from 230 µg kg^{-1} in genotype 4 (Cauamé) to 430 µg kg^{-1} in genotype 24 (MNC04-795F-158; Fig. 4). Similar results, indicating higher Se concentrations in roots than in above ground tissues in plants that did not receive Se fertiliser were observed in a study comparing two genotypes of *Prunus* rootstock, in which Se concentrations in roots varied from 260 µg kg^{-1} to 330 µg kg^{-1} dry weight, whilst in twigs and leaves, Se concentration varied from 10 µg kg^{-1} to 130 µg kg^{-1} dry weight and from 100 µg kg^{-}

^{1} to 120 µg kg^{-1} dry weight, respectively [33].

The Se concentrations in tissues of cowpea plants after the application of Se fertiliser varied widely between genotypes (Fig. 2, 3 and 4). The application of Se fertiliser increased Se concentration in grains, leaves + stems and roots of all the genotypes evaluated (Fig. 2, 3 and 4). Grain Se concentration in plants treated with Se ranged from 549 µg kg^{-1} dry weight (genotype 16; BRS Xique-Xique) to 2462 µg kg^{-1} dry weight (genotype 5; BRS Guariba). In all the genotypes evaluated, control plants had larger Se concentrations in roots than in grains, whereas, when Se fertiliser was applied, genotypes in groups a, b and c had larger Se concentrations in grain (Fig. 4) than in roots (Fig. 2), the only exception being genotype 28 (Pingo de ouro 2), which had the largest Se concentration in roots (Fig. 4). Selenium partitioning to grains also increased substantially, from 2.3% in control plants to 20% in plants receiving Se fertiliser, on average (Table A.5). This is indicative of a high Se translocation efficiency from roots to grains when Se availability is high, particularly in the genotypes from groups a, b and c for grain Se concentration (Fig. 2). Root Se concentrations in plants receiving Se fertiliser ranged from 550 µg kg^{-1}dry weight (genotype 21; MNC04-782F-108) to 1755 µg kg^{-1} dry weight (genotype 28; Ping de ouro 2).

The greater increase of Se concentration in grain than in roots of cowpea genotypes when Se fertiliser is applied might be related to factors that facilitate sodium selenate translocation from roots to grains. Sodium selenate is a readily available source of Se for plants because it does not form insoluble salts in the soil solution and is converted to organic Se compounds slowly [35]. Selenate is transferred from root to shoot tissue via the xylem and then to reproductive organs via the phloem [10,36,37]. It has been observed, in tomato, that selenate concentrations in the xylem are 7 to 13 times larger than in external media, indicating the ability of plants to transport selenate readily across roots to the xylem [38].

In the present study, the total Se in the natural soil (45 µg kg^{-1}) was larger than the Se fertiliser added as a treatment (12.5 µg kg^{-1}), although it is still a very small soil Se concentration, since soils with less than 1000 µg kg^{-1} are considered Se deficient [39]. Indeed, in soil with Se concentration

of 183 μg kg^{-1} the application of Se increased the availability of the element to maize plants [40]. Tissue Se concentrations in control plants were relatively small and the application of Se as sodium selenate caused a substantial increase in Se concentration in cowpea tissues (Fig. 2, 3, 4). This suggests that much of the Se in the natural soil was unlikely to be available to plants, while the applied Se was likely to be highly available for uptake and subsequent translocation to grains. The applied selenate is more available to plants, less adsorbed in the soil, and has been shown to influence Se accumulation in cowpea more readily than selenite, another Se source [28].

The concentration of Se in leaves + stems of cowpea when Se fertiliser was applied varied from 195 μg kg^{-1} dry weight in genotype 27 (Pingo de Ouro 1-2) to 1242 μg kg^{-1} dry weight in genotype 5 (BRS Guariba). When Se fertiliser was applied, the concentration of Se in grain was greater than in leaves + stems in all genotypes. Larger concentrations of Se in grains than in vegetative tissues was previously observed in cowpea by Silva et al [28]. By contrast, it was observed that Se concentrations in wheat tended to be larger in flag leaves than in grains [41,42] and shoot Se concentrations were also larger than grain Se concentration in rice [23].

Total grain Se concentration has also been reported to be larger in dicots than in monocots [36]. In the present study, Se concentrations in grain of cowpea genotypes ranged from 549 μg kg^{-1} to 2462 μg kg^{-1} dry weight when Se fertiliser was applied at 12.5 μg Se kg^{-1} soil (Fig. 4). In three common bean genotypes grain Se concentration varied from 2000 μg $kg^{-1}$to 4000 μg $kg^{-1}$when 5 μM Se was applied in 10 L pots in nutrient solution [34], while in two rice cultivars, grain Se concentration was less than 1 mg kg^{-1} when 500 μg Se kg^{-1} soil was applied [23] and in a study comparing 20 wheat lines, Se concentration in grains was less than 500 μg kg^{-1} when 10 mM Se was applied in 10L pots nutrient solution [42].

Data for the partitioning of Se to shoots and grains provide valuable information on the ability of genotypes to accumulate Se in edible tissues. As observed in the decision matrix (Table A.5), genotype 5 (BRS Guariba) had the largest Se concentration in leaves + stems (Fig. 3) and grain (Fig. 4), and the highest partitioning to shoots (Fig. 5). The high partitioning of Se to shoots (Fig. 5), and

the small Se concentration in roots (Fig. 2), of BRS Guariba indicate that this genotype has a high capacity to translocate Se from roots to leaves + stems and to grains, leading to a higher Se biofortification efficiency than other genotypes. Nevertheless, other genotypes had greater Se partitioning to grain than BRS Guariba (Fig. 6). For instance, genotype 24 (MNC04 795F 158), which had a larger Se partitioning to grain than BRS Guariba, had slightly smaller shoot and grain Se concentrations than BRS Guariba but a larger grain dry weight (Fig. 1c). Genotype 24 (MNC04 795F 158) had one of the largest concentrations of Se in roots among the genotypes studied (Fig. 2) and also a large Se concentration in leaves + stems and grain (Fig. 3 and 4). Genotypes such as 9 (BRS Milênio), 16 (BRS Xique xique), 21 (MNCO4 782F 108), 22 (MNCO4 792F 143) and 27 (Pingo de ouro 1 2) had among the smallest Se concentrations in roots, leaves + stems and grains under Se application (Fig. 2, 3 and 4), which indicates that the acquisition of Se by roots is relatively inefficient compared to other genotypes, although Se transport to shoots and grain is not necessarily the smallest in these genotypes (Fig. 5 and 6).

5. Conclusion

The screening of cowpea genotypes is important for selecting and breeding genotypes with a high potential for Se biofortification of grain, either though agronomic or genetic approaches. Comparative studies of Se acquisition, partitioning and accumulation in edible portions among cowpea genotypes, or even genotypes of pulses in general, are scarce. This study provides information about how each of the 29 genotypes selected accumulate Se in its grains, as well as how of the Se partitioning occurs in the whole plant. The information obtained is an important resource for selecting better genotypes for breeding programs aiming to increase the concentrations of Se in human and animal diets.

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FIGURES

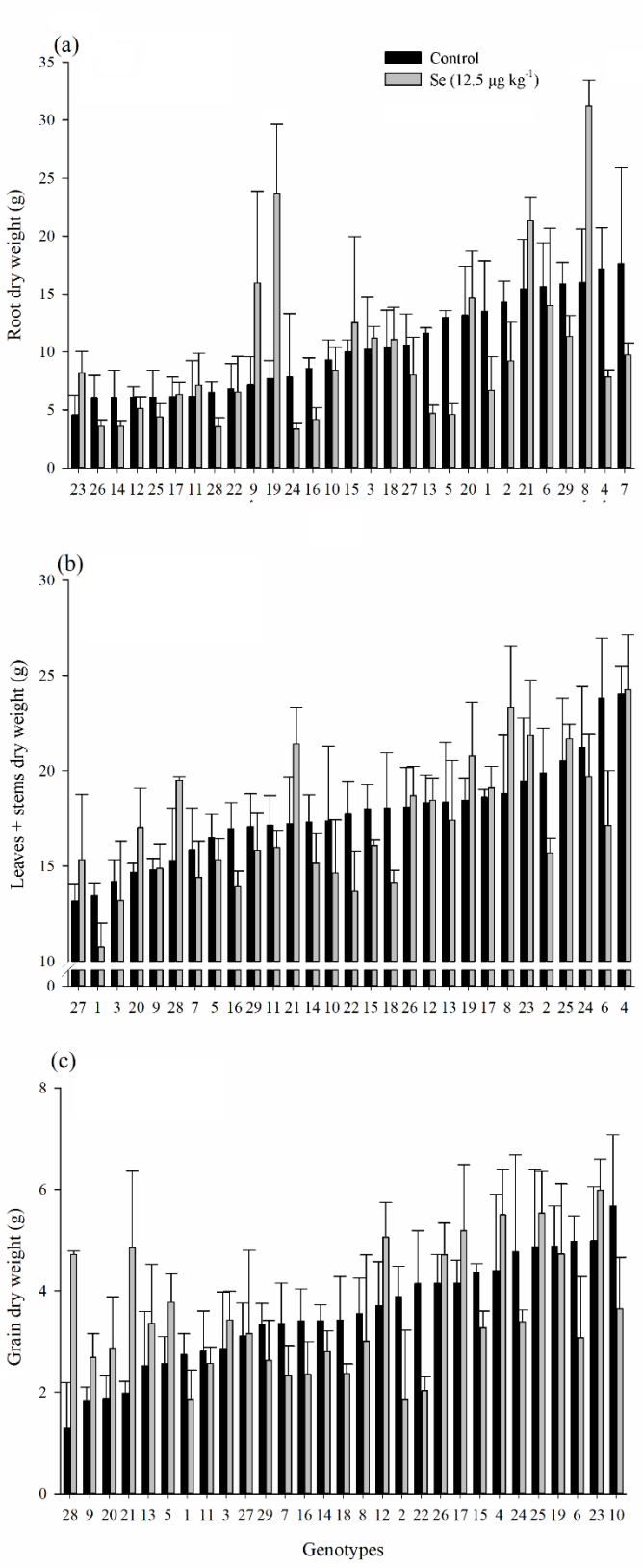

Fig. 1. Root (a), leaves + stems (b) and grain (c) dry weight of cowpea genotypes with and without application of Se. Error bars indicates the standard error of mean (n=3). CV (%) = 55.28 (a), 21.20 (b) and 44.00 (c). '*' Below numbers indicates difference between means of the same genotype under absence or presence of Se application according to the Scott-Knott test ($p \leq 0.05$).

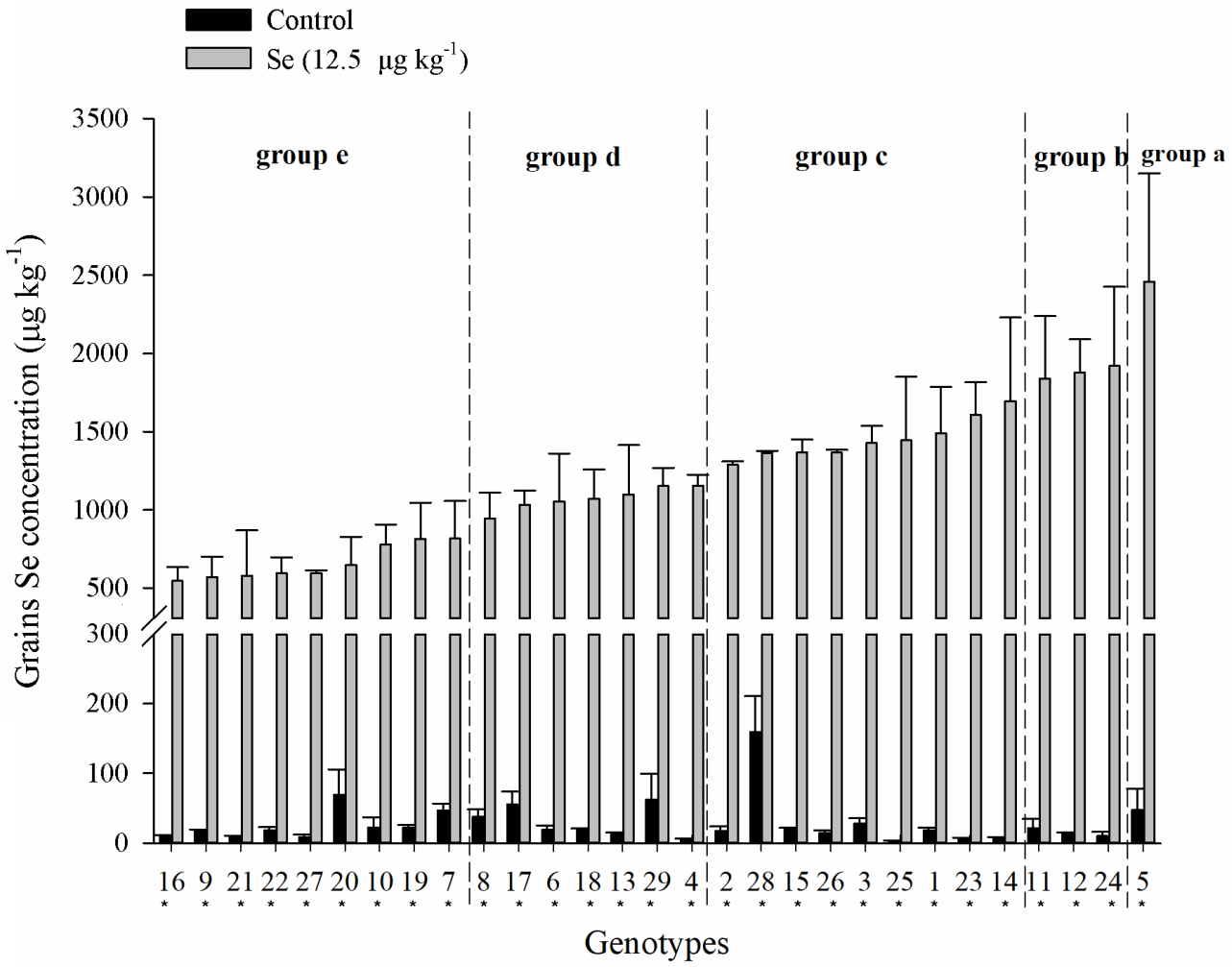

Fig. 2. Grain Se concentration in cowpea genotypes with and without application of Se. Error bars indicates the standard error of mean (n=3). CV (%) = 53.93. '*' Below numbers indicates difference between means of the same genotype under absence or presence of Se application according to the Scott-Knott test, different letter groups indicate difference among means of distinct genotypes under Se application ($p \leq 0.05$).

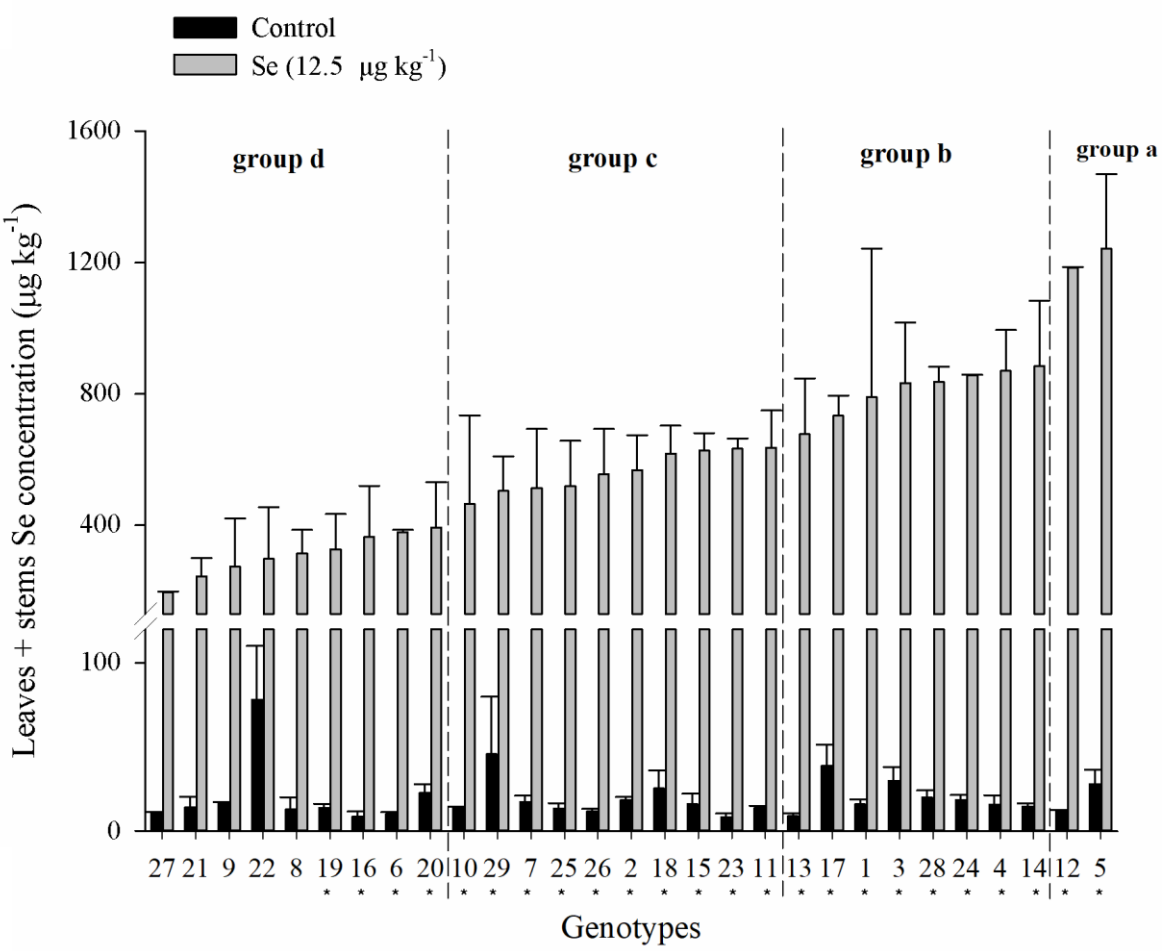

Fig. 3. Leaves + stems Se concentration in cowpea genotypes with and without application of Se. Error bars indicates the standard error of mean (n=3). CV (%) = 60.84. '*' Below numbers indicates difference between means of the same genotype under absence or presence of Se application according to Scott-Knott test, different groups indicate difference among means of distinct genotypes under Se application ($p \leq 0.05$).

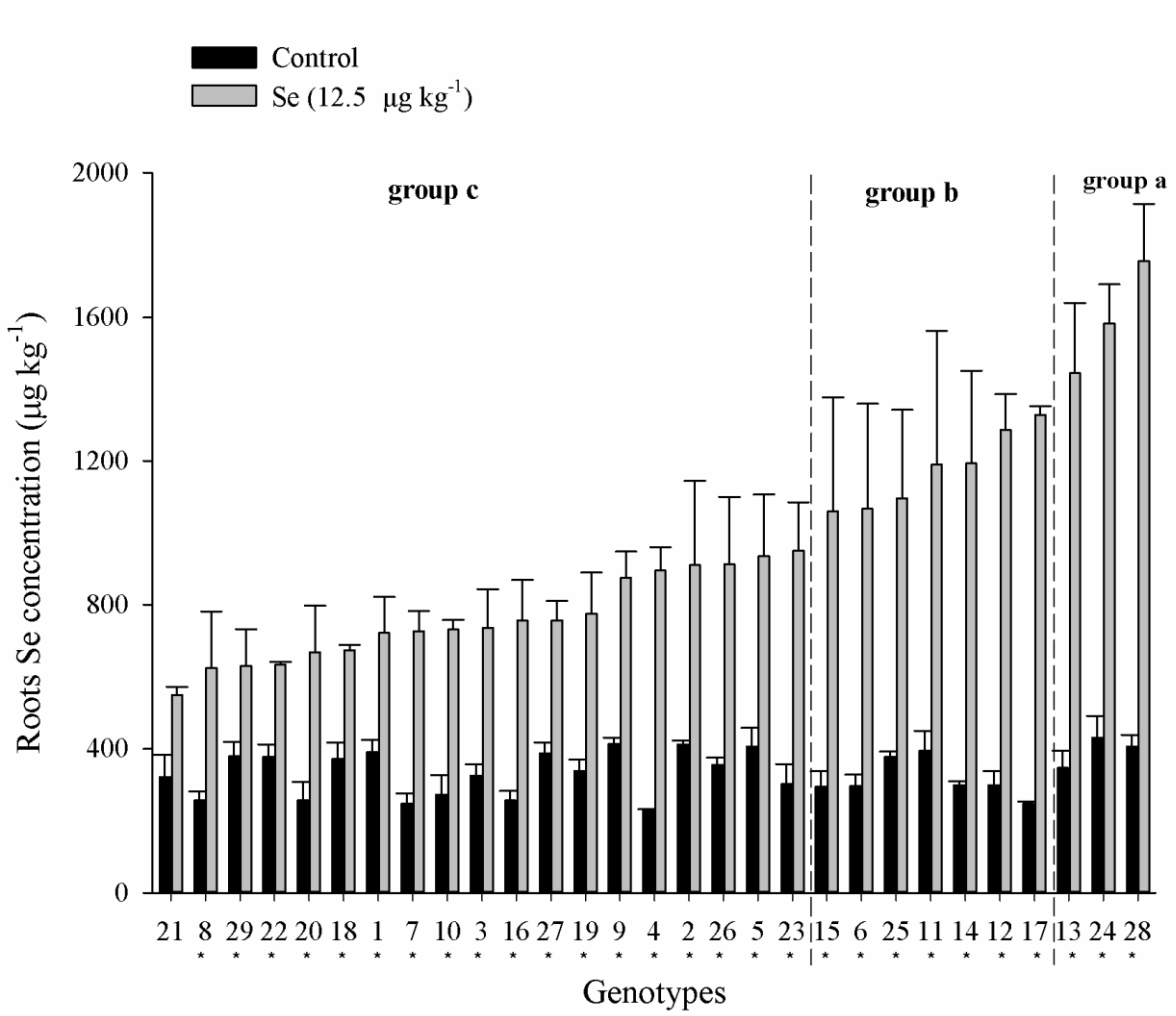

Fig. 4. Root Se concentration in cowpea genotypes with and without application of Se. Error bars indicates the standard error of mean (n=3). CV (%) = 32.37. '*' Below numbers indicates difference between means of the same genotype under absence or presence of Se application according to the Scott-Knott test, different groups indicate difference among means of distinct genotypes under Se application ($p \leq 0.05$).

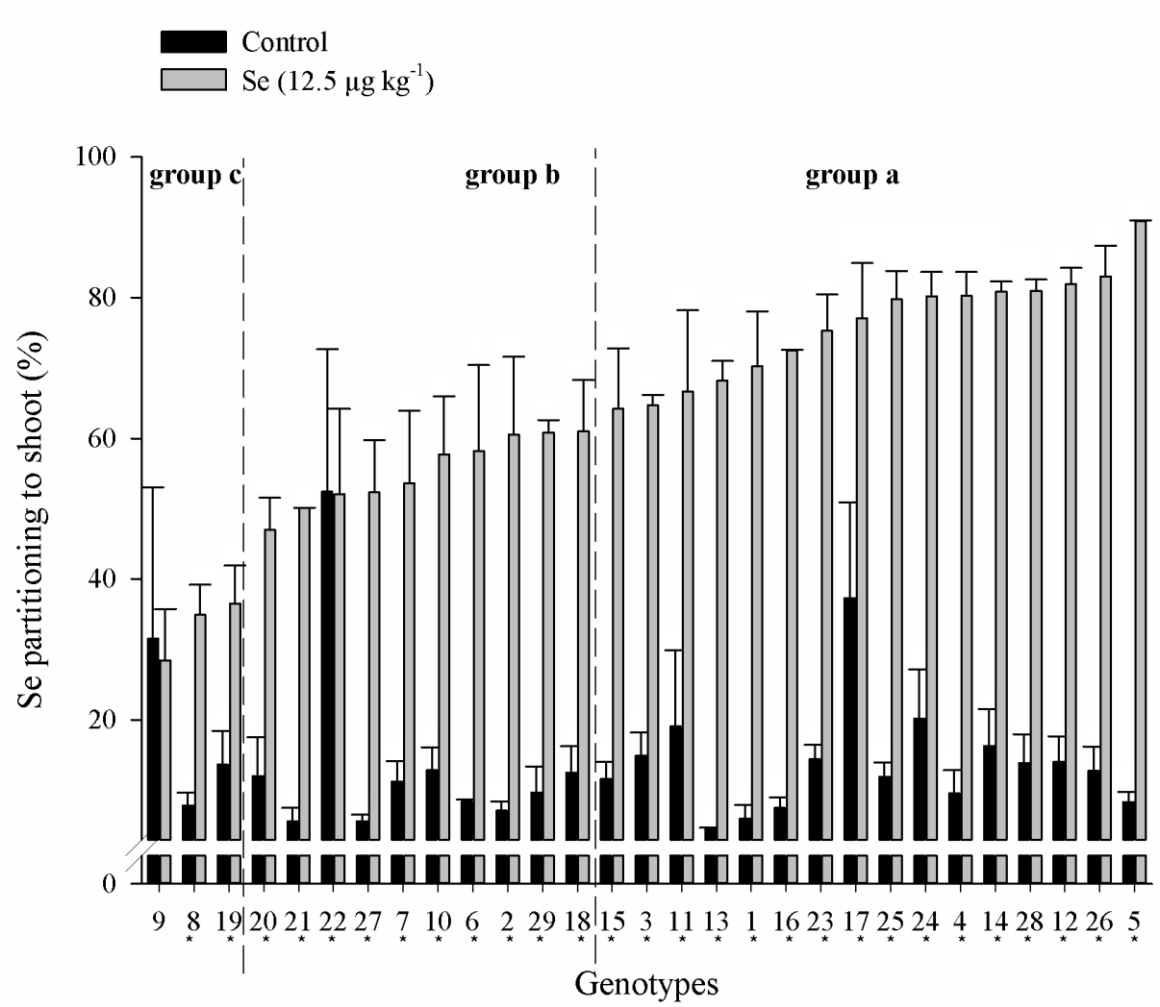

Fig. 5. Selenium partitioning to shoot in cowpea genotypes with and without application of Se. Error bars indicates the standard error of mean (n=3). CV (%) = 30.06. '*' Below numbers indicates difference between means of the same genotype under absence or presence of Se application according to the Scott-Knott test, different groups indicate difference among means of distinct genotypes under Se application ($p \leq 0.05$).

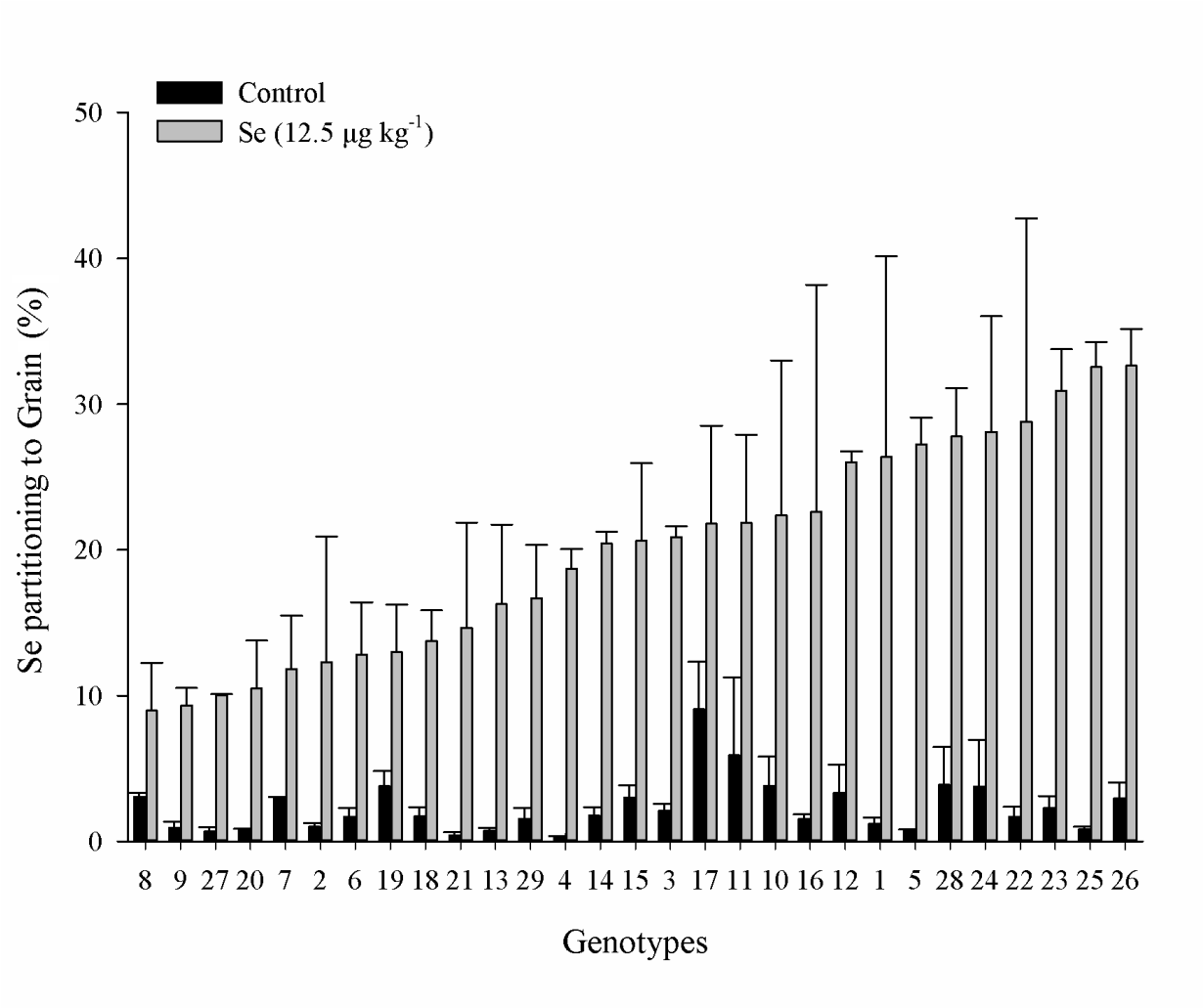

Fig. 6. Selenium partitioning to grains in cowpea genotypes with and without application of Se. Error bars indicates the standard error of mean (n=3). CV (%) = 72.10.

APPENDICES MATERIAL – TABLES

Table A.1. F ratio and P value of analysis of covariance (ANCOVA). Sources of variation: Genotypes, Se application and the variation between the factors.

Root dry weight		
Source	F ratio	Pr (<F)
Genotypes (A)	4.01	0.0001**
Se application (B)	0.53	0.47^{NS}
A X B	1.82	0.01*
Leaves + stem dry weight		
Source	F ratio	Pr (<F)
Genotypes (A)	2.98	0.0001**
Se application (B)	1.01	0.30^{NS}
A X B	0.79	0.76^{NS}
Grains		
Source	F ratio	Pr (<F)
Genotypes (A)	2.06	0.004**
Se application (B)	0.00	0.95^{NS}
A X B	1.21	0.33^{NS}
Root Se concentration		
Source	F ratio	Pr (<F)
Genotypes (A)	3.72	0.0001**
Se application (B)	379.90	0.0001**
A X B	3.13	0.0001**
Stem + Leaves Se concentratio		
Source	F ratio	Pr (<F)
Genotypes (A)	3.01	0.0001**
Se application (B)	411.52	0.0001**
A X B	3.03	0.0001**
Grains Se concentration		
Source	F ratio	Pr (<F)
Genotypes (A)	3.23	0.0001**
Se application (B)	544.69	0.0001**
A X B	3.22	0.0001**
Se partitioning to Shoot		
Source	F ratio	Pr (<F)
Genotypes (A)	3.43	0.0001**
Se application (B)	785.63	0.0001**
A X B	4.2	0.0001**
Se partitioning to Grains		
Source	F ratio	Pr (<F)
Genotypes (A)	1.47	0.08^{NS}
Se application (B)	209.96	0.0001**
A X B	1.23	0.21^{NS}

Table A.2. Mean comparison of roots dry weight of cowpea genotypes with and without application of Se, and leaves + stems (L+S) and grains dry weight of cowpea genotypes. Different letters indicate difference between means according to Scott Knott test ($p \leq 0.05$). Uppercase letters correspond to absence or presence of Se application, lowercase letters correspond to genotypes.

Treatment	Root DW (g)		L+S DW (g)	Grain DW (g)
	Control	Se		
Genotype 1	13.52 a	6.72 c	12.09 b	2.3 b
Genotype 2	14.3 a	9.2 c	17.79 b	2.88 b
Genotype 3	10.23 b	11.2 c	13.69 b	3.14 b
Genotype 4	17.17 aA	7.83 cB	24.15 a	4.95 a
Genotype 5	12.97 a	4.6 c	15.89 b	3.17 b
Genotype 6	15.63 a	14.02 c	20.46 a	4.02 a
Genotype 7	17.63 a	9.73 c	15.12 b	2.84 b
Genotype 8	16 aB	31.22 aA	21.04 a	3.28 b
Genotype 9	7.17 b	15.94 b	14.83 b	2.27 b
Genotype 10	9.33 b	8.42 c	16 b	4.66 a
Genotype 11	6.17 b	7.13 c	16.54 b	2.69 b
Genotype 12	6.1 b	5.13 c	18.39 a	4.38 a
Genotype 13	11.58 a	4.71 c	17.88 b	2.94 b
Genotype 14	6.08 b	3.57 c	16.22 b	3.11 b
Genotype 15	10 b	12.53 c	16.92 b	3.82 a
Genotype 16	8.55 b	4.16 c	15.45 b	2.89 b
Genotype 17	6.14 b	6.33 c	18.86 a	4.67 a
Genotype 18	10.41 b	11.07 c	16.09 b	2.9 b
Genotype 19	7.7 bB	23.65 bA	19.63 a	4.81 a
Genotype 20	13.17 a	14.62 c	15.84 b	2.37 b
Genotype 21	15.43 a	21.32 b	19.3 a	3.42 b
Genotype 22	6.83 b	6.54 c	15.7 b	3.36 b
Genotype 23	4.57 b	8.2 c	20.66 a	5.49 a
Genotype 24	7.83 b	3.33 c	20.46 a	4.32 a
Genotype 25	6.13 b	4.4 c	21.08 a	5.2 a
Genotype 26	6.07 b	3.57 c	18.4 a	4.43 a
Genotype 27	10.59 b	8.01 c	15.15 b	3.28 b
Genotype 28	6.52 b	3.53 c	17.41 b	3 b
Genotype 29	15.88 a	11.32 c	16.44 b	2.98 b

Table A.3. Mean comparison of Se concentration in cowpea genotypes roots, leaves+stems (L+S) and grains with and without application of Se. Different letters indicate difference between means according to Scott-Knott test ($p \leq 0.05$). Uppercase letters correspond to absence or presence of Se application, and lowercase letters correspond to genotypes.

Treatment	Roots (µg kg^{-1})		L+S (µg kg^{-1})		Grains (µg kg^{-1})	
	Control	Se	Control	Se	Control	Se
Genotype 1	390.86 c	722.02 c	16.02 aB	790.95 bA	18.99 aB	1494.33 cA
Genotype 2	411.56 bB	910.9 cA	18.45 aB	568.23 cA	18.03 aB	1293.1 cA
Genotype 3	325.22 dB	736.16 cA	29.93 aB	833.53 bA	28.35 aB	1430.99 cA
Genotype 4	230.02 eB	896.14 cA	15.67 aB	870.17 bA	4.31 aB	1160.43 dA
Genotype 5	406.36 bB	934.99 cA	27.95 aB	1242.98 aA	47.71 aB	2462.09 aA
Genotype 6	297.29 eB	1066.91 bA	10.33 aB	379.01 dA	19.21 aB	1058.9 dA
Genotype 7	247.54 eB	726.64 cA	17.53 aB	514.03 cA	47.29 aB	820.09 eA
Genotype 8	256.84 eB	624.59 cA	12.83 a	313.47 d	38.24 aB	949.81 dA
Genotype 9	413.82 bB	874.64 cA	17.16 a	273.55 d	17.98 aB	573.68 eA
Genotype 10	272.11 eB	731.33 cA	13.69 aB	465 cA	22.97 aB	781.73 eA
Genotype 11	394.88 cB	1190.31 bA	13.94 aB	636.5 cA	21.88 aB	1844.02 bA
Genotype 12	298.7 dB	1285.98 bA	11.68 aB	1183.51 aA	13.88 aB	1881.77 bA
Genotype 13	347.07 dB	1444.42 aA	9 aB	677.63 bA	13.36 aB	1103.5 dA
Genotype 14	299.05 dB	1193.79 bA	14.69 aB	885.14 bA	8.59 aB	1699.38 cA
Genotype 15	295.53 eB	1059.64 bA	16.17 aB	628.18 cA	20.96 aB	1371.28 cA
Genotype 16	256.64 eB	756.63 cA	8.66 aB	363.63 dA	10.49 aB	549.72 eA
Genotype 17	246.89 eB	1327.85 bA	38.8 aB	733.42 bA	55.73 aB	1037.78 dA
Genotype 18	372.21 c	674.02 c	25.41 aB	618.55 cA	19.13 aB	1073.24 dA
Genotype 19	338.04 dB	774.97 cA	13.85 aB	326.4 dA	22.12 aB	818.5 eA
Genotype 20	256.62 eB	668.38 cA	22.84 aB	392.57 dA	69.46 aB	652.16 eA
Genotype 21	322.23 d	550.08 c	14.24 a	243.91 d	8.6 aB	582.12 eA
Genotype 22	377.46 c	633.92 c	78.16 a	297.08 d	19.03 aB	597.59 eA
Genotype 23	301.91 dB	951.69 cA	8.42 aB	633.18 cA	6.15 aB	1613.25 cA
Genotype 24	430.65 aB	1582.07 aA	18.67 aB	857.11 bA	10.43 aB	1924.42 bA
Genotype 25	378.34 cB	1096.58 bA	13.4 aB	518.77 cA	3.59 aB	1448.57 cA
Genotype 26	355.62 cB	913.32 cA	11.8 aB	555.07 cA	15.03 aB	1371.66 cA
Genotype 27	387.07 cB	757.42 cA	11.24 a	195.29 d	8.85 aB	598.96 eA
Genotype 28	405.32 cB	1755.7 aA	20.03 aB	837.32 bA	159.91 aB	1368.87 cA
Genotype 29	378.91 c	630.35 c	45.85 aB	504.77 cA	62.92 aB	1157.27 dA

Table A.4. Mean comparison of Se partitioning to shoot of cowpea genotypes with and without application of Se. Different letters indicate difference between means according to Scott Knott test ($p \leq 0.05$). Uppercase letters correspond to absence or presence of Se application, lowercase letters correspond to genotypes.

Treatment	Se partitioning to shoot (%)	
	Control	Se
Genotype 1	05.99 bB	70.28 aA
Genotype 2	07.09 bB	60.52 bA
Genotype 3	14.91 bB	64.75 aA
Genotype 4	09.54 bB	80.31 aA
Genotype 5	09.12 bB	90.92 aA
Genotype 6	08.37 bB	58.22 bA
Genotype 7	11.25 bB	53.66 bA
Genotype 8	07.86 bB	34.91 cA
Genotype 9	32.33 a	28.46 c
Genotype 10	12.85 bB	57.77 bA
Genotype 11	19.07 bB	66.69 aA
Genotype 12	14.02 bB	81.93 aA
Genotype 13	04.54 bB	68.27 aA
Genotype 14	16.32 bB	80.90 aA
Genotype 15	11.59bB	64.27 aA
Genotype 16	07.48 aB	72.50 aA
Genotype 17	37.29 bB	77.10 aA
Genotype 18	12.51 bB	61.00 bA
Genotype 19	13.65 bB	36.54 cA
Genotype 20	12.72 bB	47.07 bA
Genotype 21	05.54 bB	50.16 bA
Genotype 22	52.43 b	52.09 b
Genotype 23	14.39 bB	75.28 aA
Genotype 24	20.20 bB	80.23 aA
Genotype 25	11.90 bB	79.80 aA
Genotype 26	12.73 bB	83.04 aA
Genotype 27	05.62 bB	52.36 bA
Genotype 28	15.05 bB	81.00 aA
Genotype 29	10.46 bB	60.84 bA

Treatment	Se translocation to grains (%)
Se Application	64.51 A
Control	14.22 B

Table A.4. Mean comparison of Se partitioning to grains of cowpea genotypes. Different letters indicate difference between means according to Scott Knott test ($p \leq 0.05$). Uppercase letters correspond to absence or presence of Se application, lowercase letters correspond to genotypes.

Treatment	Se partitioning to grains (%)
Genotype 1	13.80 a
Genotype 2	06.64 b
Genotype 3	11.49 a
Genotype 4	09.50 b
Genotype 5	13.97 a
Genotype 6	07.25 b
Genotype 7	07.43 b
Genotype 8	06.01 b
Genotype 9	05.13 b
Genotype 10	13.08 a
Genotype 11	13.87 a
Genotype 12	14.68 a
Genotype 13	08.50 b
Genotype 14	11.10 a
Genotype 15	11.83 a
Genotype 16	12.08 a
Genotype 17	15.43 a
Genotype 18	07.73 b
Genotype 19	08.40 b
Genotype 20	05.68 b
Genotype 21	07.54 b
Genotype 22	15.25 a
Genotype 23	16.61 a
Genotype 24	15.93 a
Genotype 25	16.55 a
Genotype 26	17.79 a
Genotype 27	05.35 b
Genotype 28	15.84 a
Genotype 29	09.11 b
Treatment	**Se translocation to grains (%)**
Se application	19.99 A
Control	2.32 B

Table A.5. Decision Matrix for variables presenting Se and Genotype interaction. The "*" indicates genotypes that presented the highest result observed, for each specific variable.

Variable	Genotypes	
	5 (BRS Gruariba)	28 (Pingo-De-Ouro-2)
Grain Se Concentration	*	
Leaves + Stem Se Concentration	*	
Roots Se Concentration		*
Se Partitioning to Shoots	*	

APPENDICES MATERIAL – FIGURES

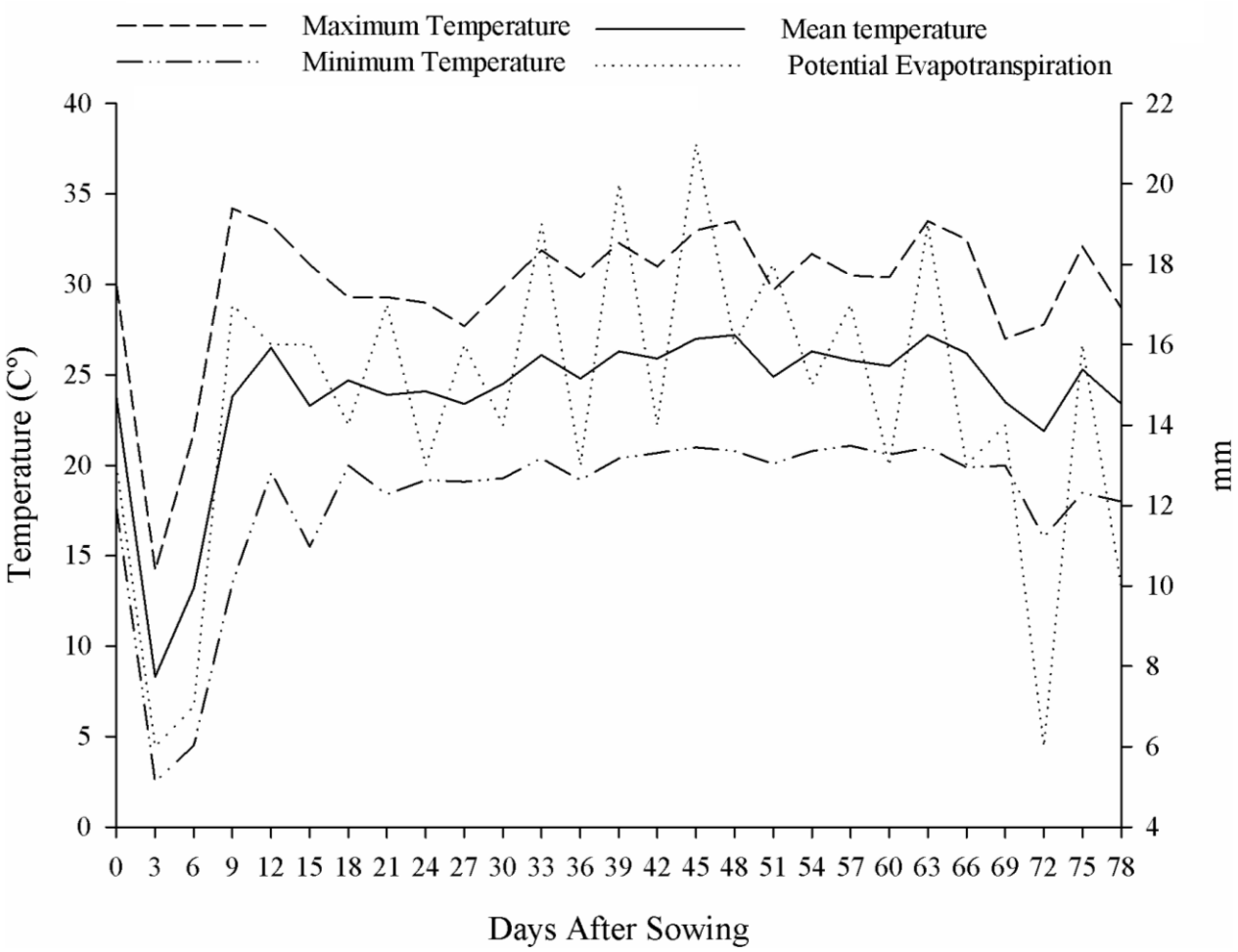

Figure A.1. Maximum, mean and minimum temperature, and potential evapotranspiration during cowpea greenhouse experiment.