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Red Kidney Bean (*Phaseolus vulgaris L.*) Germination and Seedling Growth as affected by Selenium, Nano- Selenium and Sulfur

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Abstract

Selenium and sulfur similarity in chemical properties and transport pathways through plant pretend that they may share or compete in some metabolic activities during seed germination and plant growth. This work aimed to study the effect of soaking red kidney bean seeds (*Phaseolus vulgaris L.*) for 2h in aerated solution of Gum Arabic-Coated Selenium Nanoparticles (GA-SeNPs ≈ 48.22 nm), Sodium Selenate (Na_2SeO_4) and Sodium Sulphate (Na_2SO_4), each at 0.0, 0.5, 1, 5, 10, 25, 50 µM concentrations, on germination and seedling growth. The control and the treated seeds were germinated at 25°C ± 0.5 under dark controlled conditions for 4 days. GA-SeNPs, Na2SeO4 and Na2SO4 significantly improved germination percentage and seedling growth criteria of red kidney bean up to 10 µM, as compared with the controls. Red kidney bean responded in more or less similar trend to the different concentrations of the treatment solutions, with the magnitude of improvement was always greater for GA-SeNPs than for Na_2SeO_4 and Na_2SO_4. Moreover, both Na_2SeO_4 and Na_2SO_4 significantly decreased germination percent and seedling growth at 50 µM than the controls.

The results indicated the successful use of GA-SeNPs up to 50 µM, Na_2SO_4 and Na_2SeO_4 up to 5 µM, for enhancing the germination potential and subsequent seedling growth of red kidney bean under study.

Keywords: Red kidney bean seeds (*Phaseolus vulgaris L.*); Selenium nanoparticles; Sodium selenate; Sodium sulphate; Germination; Seedling growth

Introduction

Common beans are members of *Fabaceae* family, which are known for it's economic and nutritional values. Red kidney bean (*Phaseolus vulgaris L.*) variety has low-fat, high protein contents and contains several bioactive compounds [1]. Red kidney bean is one of the most globally important legumes and considered as an essential component of human nutrition due to its high protein content (20%-25%), complex sugars (50%-60%), vitamins especially vitamin E, minerals, poly-unsaturated fatty acids and tangible amounts of folate and fibers (4%) [2-5]. It also contains a large number of phytochemical components including phenolic, flavonoids, vitexin and isovitexin [6]. It has the highest antioxidant activity compared with other types of legumes [7].

Selenium (Se) is essential for humans and animals, and its ingestion is associated with risk reduction of numerous diseases, such as cancer [8]. However, nearly 1 billion people in the world suffer from Se deficiency due to the consumption of foods with low Se concentration [9], especially as a result of the low and uneven availability of Se in most soils around world [10]. Considering the imbalance of Se contents in soils and those plants are primary sources for the entry of Se into the food chain, agronomic bio-fortification through the use of concentrated fertilizers is one of the primary alternatives to increase the bioavailability of Se in food [11]. The application of low concentrations of Se has been reported to have beneficial effects on crops. For example, Se application increased antioxidant activity and consequently increased yields [12, 13].

Recently, nanotechnology has been extensively employed in the field of plant sciences to explore its potential impacts in improving crop yields, selenium nanoparticles (SeNPs), have aroused worldwide attention due to their distinguished properties and excellent biological activities [14]. It is able to scavenge free radicals *in vitro* [15] and stimulate organogenesis [16]. Nano-Se has a higher efficiency in up regulating selenoenzymes and exhibits less toxicity compared with other Se compounds such as selenite [17], selenomethionine [18] and Se-methyl selenocysteine [19]. A new approach to fertilization of plants is the use of selenium nanomaterials [20].

Similarity between selenium and sulfur was proved in chemistry so, it is important to study the stimulatory or inhibitory action of both elements related to plant minerals nutrition and growth. Se and sulfur (S) are both group-16 "chalcogens" in the periodic table, meaning they have similar ionic radii, covalent radii, and chemical properties [21]. Indeed, selenate enters plant roots using sulphate transporters [22]. Considering S chemical similarity with Se, S forms tend to compete in such processes as absorption, translocation, and assimilation. For example, when Se is present as selenate, it enters the same pathway as sulphate, replacing it in the synthesis of such proteins as cystine and methionine [23, 22].

Therefore, the S compounds present in the rhizosphere may inhibit Se uptake by plants [24-26], thus influencing levels of these elements in plant tissues. Sulfur is also well known as an important macroelement, it plays a key role in the nutrition and production of agricultural crops. Sulphate taken up by the roots is the primary sulfur source for growth, but additionally plants are able to utilize absorbed sulfur gases by the shoot. Sulfur is a great indication for the structure of proteins and functioning of enzymes, and it plays an important role in the defense of plants against stresses and pests. Sulfur metabolites such as glutathione provide protection for plants against oxidative stress, heavy metals and xenobiotic. Humans and animals rely on plants for their reduced sulfur and plant sulfur nutrition has a decisive effect on food quality, e. g., availability of methionine [27].

The impact of selenium and sulfur on higher plants appears to depend on the species, age of plant, concentration and the experimental

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conditions. Se is a beneficial element for plants at low concentrations, but at high concentrations it is toxic. At low concentrations, Se show a promotive effect on germination percentage and physiological quality of seeds in several crops, such as barley, white mustard, oilseed rape [28], rice [29] and increases the antioxidant properties of higher plants, which reflect the reduction of reactive oxygen species [30]. In addition, Low concentration of Nano-Se and Se, improved tomato growth parameters and chlorophyll content under high and low temperature stress [31] and enhanced seedling growth and hydrolytic enzymatic activity in germinated cowpea seeds up to 25 µM [32]. On the contrary, high concentrations of Se reduced seed germination in bitter melon [33], *Arabidopsis thaliana* plants [34], inhibit germination and may lead to the death of the embryo in rice by inactivating hydrolytic carbohydrate enzymes [29]. Exposure to high Se (800 mg L^{-1}); decreased seed germination and seedling growth, which reflect the increase of total sugars and sucrose concentration in both shoot and root of *cowpea* (*Vigna unguiculata*) [35]. Sulfur plays a role in building of proteins and chlorophyll [36,37], activation of Reactive Oxygen Species (ROS) scavenging enzymes and improves antioxidant defense under abiotic stresses [38]. Indirectly, S interacts with auxins, gibberellins, cytokinin, ethylene and salicylic acid, to counteract abiotic stresses [39]. On the other hand, its deficiency regulates the chlorophyll content of leaves, N content and photosynthetic enzymes [40] and suppresses cell sap osmotic pressure [41]. However, the mechanisms that mediate the effects of selenium and sulphur in plants remain unknown. Therefore, it is important to increase our knowledge about these mechanisms before implementing large-scale agricultural utilization of selenium and sulfur.

This work, aimed to study the effect of soaking red kidney bean (*Phaseolus vulgaris L.*) in different concentrations of Gum Arabic-Coated Selenium Nanoparticles (GA-SeNPs), Sodium selenate (Na_2SeO_4) and Sodium sulphate (Na_2SO_4) on the percent of seed germination and seedlings growth criteria in seedling.

Materials and Methods

Synthesis of selenium nanoparticles

Gum Arabic (GA) Coated Selenium Nanoparticles (GA-SeNPs ≈ 48.22 nm) was synthesized and various characterization techniques (UV-vis spectroscopy, Dynamic Light Scattering (DLS), Transmission Electron Microscopy (TEM) and Fourier Transform Infrared (FT-IR) spectroscopy) confirmed the formation of phytochemicals-capped SeNPs (under publication).

Plant materials and treatments

A pure lot of red kidney bean (*Phaseolus vulgaris L.*) seeds were provided by Sakha Horticulture Research Station, Horticulture Research Institute, Agricultural Research Center, Giza, Egypt.

Uniform seeds of red kidney bean were sterilized with 2.5% sodium hypochlorite solution for three minutes, and thoroughly rinsed with distilled water until complete removal of hypochlorite. Afterwards, seeds were equally divided into three batches and each batch was subdivided into groups (100 seeds each) to be soaked in the different working solutions. Soaking of seeds was carried out by putting a constant number of seeds of each batch for 2 h in glass containers, each containing a constant amount (100 ml) of the treatment solutions (Gum Arabic-Coated Selenium Nanoparticles (GA-SeNPs), sodium selenate and sodium sulphate) each at concentrations of (0.5, 1, 5, 10, 25, 50 µM). In addition, distilled water was used as a control. Soaking was carried out in a controlled cabinet (germinator). Afterwards, the seeds of the control and each treatment were washed thoroughly with distilled water, and then allowed to germinate in sterilized petri-dishes containing filter paper wetted with about 5 ml of distilled water (6 replicates, each contains 10 seeds) for 4 days. Germination was carried out in a controlled cabinet (incubator) at 25C° ± 0.5 under dark conditions.

Growth measurement

The germination percentage (%) was calculated at the 4th day, according to the following:

$$Germination\% = \frac{No.\,of\ Germinated\ Seeds}{Total\ no.\,of\ Seeds} \times 100$$

For measurements of different growth criteria (plumule and radicle length (cm), fresh and dry weights (g) per seedling), at least 12 randomly choice 4-day-old seedlings were taken from each treatment and the control. The collected samples were dried in an oven at 75°C until constant dry weight was obtained.

Statistical analysis

The data was expressed as mean of six replicates; each replicate consist of two seedlings. Statistical Analysis of the data was carried out using one-way Analysis of Variance (ANOVA) using least significant difference (LSD at 5% level) followed by Duncan's Multiple Comparison Test.

Results

Germination percentage

Data presented in Table 1, show the germination percentage of the control (H_2O) and the differently treated red kidney bean (*Phaseolus vulgaris L.*).

The germination percentage was generally significantly enhanced, as compared to control, in response to soaking for 2 hours in different concentrations of GA-SeNPs, Na_2SeO_4 and Na_2SO_4 at low concentrations (up to 10 µM), and then progressively decreased at higher concentrations (but still higher than control at 25 µM GA-SeNPs).

The best results were obtained in response to soaking seeds in solutions of GA-SeNPs at 1 µM and Na_2SO_4 at 5 µM, where, germination percent was improved by 21.36 and 17.99%, respectively relative to control.

However, at high concentration (50 µM) GA-SeNPs, Na_2SeO_4 and Na_2SO_4 negatively affected the germinative power in the order of 8.98, 15.05 and 21.12%, respectively in comparison to the control treatment (Table 1).

Growth criteria of seedling

Table 1 shows the main growth criteria of 4-day-old seedlings resulting from pre-sowing seed soaking for 2 hours in different concentrations of GA-SeNPs, Na_2SeO_4 and Na_2SO_4.

In case of the three applied treatments (nano-selenium, sodium selenate and sodium sulphate), the growth criteria (plumule and radicle lengths, fresh and dry weights) of seedlings were mostly significantly increased, relative to corresponding controls, from

Table 1: Percentage of germination and growth criteria of 4 day-old seedlings (germinated in dark at 25°C ± 0.5) of red kidney bean (*Phaseolus vulgaris L.*) as affected by seed pre-sowing for 2h in gum arabic-coated selenium nanoparticles (GA-SeNPs), Na_2SeO_4 and Na_2SO_4. Each value represents the mean of 6 replicates for growth criteria. Different letters indicate significant differences between treatments (Duncan test, P ≤ 0.05). Means, in each column, followed by similar letters are not significantly different.

Treatment µM	Germination percent	Plumule length	Radicle length	Fresh weight	Dry weight
	%	Cm	Cm	g	g
Control	82.4^{h}	3.50^{ef}	2.83^{h}	1.29^{bcd}	0.39^{ab}
Na_2SeO_4 0.5	85.0^{g}	3.63^{ef}	2.90^{h}	1.34^{bcd}	0.41^{ab}
Na_2SeO_4 1	90.0^{f}	3.90^{de}	3.20^{g}	1.36^{bcd}	0.46^{a}
Na_2SeO_4 5	90.0^{f}	5.23^{b}	4.60^{e}	1.48^{b}	0.47^{ab}
Na_2SeO_4 10	90.0^{f}	4.63^{c}	4.43^{e}	1.38^{bc}	0.41^{ab}
Na_2SeO_4 25	82.4^{h}	3.40^{ef}	2.57^{i}	1.29^{bcd}	0.37^{ab}
Na_2SeO_4 50	70.0^{j}	3.00^{fg}	2.43^{j}	1.19^{cde}	0.35^{ab}
GA-SeNPs 0.5	92.00^{d}	4.50^{c}	5.10^{d}	1.48^{b}	0.41^{ab}
GA-SeNPs 1	100.00^{a}	6.00^{a}	7.20^{a}	1.73^{a}	0.48^{a}
GA-SeNPs 5	95.00c	6.00^{a}	5.80^{b}	1.71^{a}	0.46^{ab}
GA-SeNPs 10	90.56^{de}	5.00^{bc}	5.60^{c}	1.42^{b}	0.41^{ab}
GA-SeNPs 25	85.00^{g}	3.60^{ef}	4.50^{e}	1.37^{bcd}	0.40^{ab}
GA-SeNPs 50	75.00^{i}	3.00^{fg}	3.70^{f}	1.29^{bcd}	0.37^{b}
Na_2SO_4 0.5	90.0^{f}	3.67^{e}	3.20^{g}	1.42^{b}	0.43^{ab}
Na_2SO_4 1	95.0^{c}	4.40^{cd}	3.37^{g}	1.45^{b}	0.46^{ab}
Na_2SO_4 5	97.2^{b}	5.40^{b}	5.10^{d}	1.74^{a}	0.47^{a}
Na_2SO_4 10	90.6^{de}	3.60^{ef}	2.87^{h}	1.32^{bcd}	0.40^{ab}
Na_2SO_4 25	75.0^{i}	2.80^{g}	2.23^{j}	1.18^{e}	0.36^{ab}
Na_2SO_4 50	65.0^{k}	2.77^{g}	1.67^{k}	1.08^{e}	0.34^{ab}
LSD at 0.05	2.0	0.60	0.17	0.17	0.19

0.5 to 10 µM concentrations. In this respect, best performance was induced by 1 and 5 µM GA-SeNPs, followed by 5 µM of Na_2SO_4 and Na_2SeO_4, where nano-Se at 1 µM induced the highest significant increase in plumule length (71.43%), radicle length (154.42%), fresh weight (34.11%) and dry weights (23.08%) of seedling in comparison to the control. Furthermore, nano-Se was more efficient than sodium sulphate and sodium selenate at comparable concentrations in increasing growth criteria of red kidney bean (Table 1 and Figure 1).

On the other hand, the growth criteria of red kidney bean seedling started to decrease at 25 µM of either Na_2SeO_4 or Na_2SO_4, while a moderate decrease was observed in plumule length (14.29 and 20.86%), radicle length (14.13 and 40.99%), fresh weight (7.75 and 16.28%) and dry weights (10.26 and 12.82%) of seedling in comparison to the control at 50 µM concentration of Se and S, respectively (Table 1).

Figure 1: Seedlings growth of red kidney bean (*Phaseolus vulgaris L.*) as affected by soaking for 2h in gum arabic-coated selenium nanoparticles (GA-SeNPs) at 0.0, 0.5, 1, 5, 10, 25 and 50 µM followed by germination at 25°C ± 0.5 in dark.

Discussion

In the present study, the germination percentage and the main growth criteria (plumule and radicle lengths and fresh and dry weights) of 4-day-old red kidney bean seedlings resulting from soaking for 2 hours in different concentrations of gum arabic-coated selenium nanoparticles (GA-SeNPs), sodium selenate and sodium sulphate were mostly significantly increased, relative to controls, from 0.5 to up to 10 µM concentrations. Gum arabic-coated selenium nanoparticles stimulatory effects were more efficient than sodium sulphate and Na_2SeO_4 at comparable concentrations. The best performance was induced by GA-SeNPs at 1 and 5 µM, followed by 5 µM of Na_2SO_4 and Na_2SeO_4, where nano-Se at 1 µM induced the highest significant increase in plumule length (71.43%), radicle length (154.42%), fresh weight (34.11%) and dry weight (23.08%) of seedling relative to control (Figure 1 and Table 1). These conclusions were in alliance with those obtained with three common bean (*Phaseolus vulgaris L.*) cultivars, where rise in the concentration of $AgNO_3$ or AgNPs from 20 to 80 ppm significantly improved germination percentage, germination rate and seedling growth criteria [42], SeNPs at low concentration (2-10 mg L^{-1}) showed a positive effect on root growth and germination index of *Dorema aucheri* plant [43] and *cowpea*, in response to seed pre-soaking with Na_2SeO_4 and nano Se at 6.25-25 µM [32]. In accordance, Se at low concentrations act as an antioxidant and stimulated growth, whereas higher concentrations act as a pro-oxidant and reduced ryegrass yields [44]. Se has a role in mitochondrial membrane functions [45]; hence low concentration of Se improves respiration activity in young pea (*Pisum sativum L.*) [46]. The enhanced percentage of germination might be attributed to an increased permeability of the seed testa, thus facilitating the admission of water and di-oxygen into the cells, which would then

accelerate germination and concomitant metabolic processes [47]. In addition, the enhanced seedling growth by seed soaking in Se, nano-Se and S, particularly at lower concentrations may be due to *de novo* synthesis of certain germination-promoting substances, promotion plant cell division, elevation effect of some hydrolytic enzymes (α-amylase, β-amylase and protease) that resulted in efficient utilization of seed reserves, stimulation of antioxidant activity, and increased abilities for absorbing and utilizing water as has been concluded by [48] in cauliflower, [49] in rice, [32] in *cowpea* and [29] who found that seeds priming with Se enhanced *de novo* synthesis of germination-promoting substances, membrane re-organization, activity of hydrolytic enzymes and reduced leakage of metabolites, through increased GSH-Px activity [12, 50], as Se is present in the GSHPx enzyme active site which participates in the reduction of toxic hydrogen peroxide and lipid peroxides [51]. Se at low levels (0.1-0.75 ppm) can stimulate the shoots growth in 10-day-old mungbean seedlings by up-regulation carbohydrate metabolism enzymes, thus providing energy substrates for enhanced growth, where, the activity of starch hydrolysing enzymes (α-and β-amylases) and sucrose hydrolyzing enzyme (invertase) were stimulated significantly associated with elevation of activities of sucrose synthesising enzymes (sucrose synthase and sucrose phosphate synthase) [44]. Also, the maximum activities of α-amylase, β-amylase and protease enzymes in *cowpea* seedlings were obtained in response to soaking seeds in solutions of either Na_2SeO_4 or SeNPs at 6.25 µM [32]. Mahakham [52] proposed that different mechanisms underlying nanopriming-induced seed germination, including creation of nanopores for enhanced water uptake, stimulation the up-regulation of aquaporin genes, rebooting ROS/antioxidant systems in seeds, generation of hydroxyl radicals for cell wall loosening, and nanocatalyst for fastening starch hydrolysis compared to unprimed control and other priming treatments. With respect to sulfur, low concentrations of sodium sulphate and sodium chloride stimulated growth in *Chenopodium rubrum L.*, but higher concentrations resulted in large decreases in dry weight and leaf area [53], foliar spray with 6 ppm of sulfur enhance the shoot and root biomass accumulation, physiological and biochemical attributes and alleviate the effect of heat stress in tomato plants [54]. Sulfur and its derivatives play vital roles in the activation of Reactive Oxygen Species (ROS) scavenging enzymes to improve antioxidant defense under abiotic stresses [38]. Indirectly, S interacts with auxins, gibberellins, cytokinin, ethylene and salicylic acid, to counteract abiotic stresses [39]. Plants need thiol-containing S biomolecules to develop a defensive mechanism against different abiotic stresses [55].

On the contrary, the percentage of germination and the growth criteria of 4-day-old red kidney bean seedlings started to decrease at 25 µM of either Na_2SeO_4 or Na_2SO_4, while a moderate decrease was observed in plumule length (14.29 and 20.86%), radicle length (14.13 and 40.99%), fresh weight (7.75 and 16.28%) and dry weight (10.26 and 12.82%) of seedling in comparison to the control at 50 µM concentration of Se and S, respectively (Table 1). In consistent, [34] noticed an inhibition in germination of *Arapidopsis thaliana* species sown in soil close to Se hyper accumulator species due to their apparent ability of concentrate Se. In *cowpea*, seed germination was inhibited at high concentrations of Se [29], plumule and radicle lengths was reduced in response to soaking in Se and nano-Se at 50-100 µM [32], root growth, (seedling length, the shoot and root fresh weight) started to decrease at 1 and 40 mg L^{-1} Se concentration, respectively, while a drastic decrease was observed in shoot (74 and 82%) and root (87 and 94%) growth in comparison to the control at 400 and 800 1 mg L^{-1} Se concentrations, respectively. The drastic decrease of photosynthetic pigments, increase of total sugars and sucrose levels in shoot and root are related to the lower seedling growth development in response to high Se exposure [35]. In *Dorema aucheri* plant, SeNPs at 30 mg L^{-1} decreased seed germination index by 20%, compared to the control [43], while application of ZnONPs (25, 50, and 100 mg L^{-1}) had no obvious effects on the seed germination, however, it improved the early growth and related physio-biochemical attributes in rice [56].

In the present study, the observed decrease in red kidney bean seedling growth at an elevated concentration of either nano-Se, Se or S, may be attributed to elevated MDA levels and the activities of antioxidants in an attempt to cope with oxidative stress. Metabolic activities are expected to increase remarkably in kidney bean seeds following their soaking with nano-Se, Se or S that may lead to higher activity of ROS as secondary products of mitochondrial respiration. Additionally, [46] found higher respiratory activity associated with low doses of Se in young pea (*Pisum sativum L.*) plants. There is strong evidence that free radicals and peroxides are abundantly produced within seeds during germination [57] and are cooperatively tackled by enzymatic reactions [49]. The enhanced expression and activity of antioxidant enzymes has been proposed as part of seed strategy to cope with ROS produced during seed priming [29]. Se has been ascribed as a natural stimulant of antioxidant activity in plants [58]. Wang [59] found that low (non-toxic) Se concentrations up-regulate proteins involved in ROS detoxification and resistance to pathogens. The expression of the same proteins was down-regulated by high (toxic) Se concentrations. In two fine rice cultivars (Super and Shaheen Basmati), the activity of α-amylase was significantly induced by priming with selenium (15-60 µmol L^{-1}). Nevertheless, Se at 90 and 105 µmol L^{-1} had detrimental effect on α-amylase activity that was dropped even below than that of the control at 105 µmol L^{-1} [29]. Endogenous phytohormones that are known to balance cell proliferation are modulated by nanoparticles [60, 61]. At high concentrations, Selenium replaces sulfur in amino acids leading to malformed selenoproteins formed due to the misincorporation of selenocysteine/seleno-methionine in place of cysteine/methionine in protein chain which are nonfunctional proteins and enzymes and contributes to its toxicity [62]. Seleno-amino acids incorporated into protein may result in S-S bonds being replaced by the less stable Se-Se bonds, leading to changes in biological activity of the protein [63-65].

Conclusion

The present study indicates that soaking red kidney bean seeds in solutions of GA-SeNPs, Na_2SeO_4 and Na_2SO_4 at different concentrations (0.5-50 µM) improved germination percentage, and seedling growth criteria particularly at lower concentrations.

Generally, GA-SeNPs at 1 and 5 µM was the most effective treatments in enhancing germination and seedling growth parameters followed by Na_2SO_4 at 5 µM, then Na_2SeO_4 at the same concentration, compared to controls. A reverse situation was recorded at higher concentration 50 µM, where seedling growth criteria was the lowest in treatment with Na_2SO_4 followed by Na_2SeO_4, then GA-SeNPs. However, field studies are needed to determine the possible role of GA-SeNPs, Na_2SeO_4 and Na_2SO_4 in improving red kidney bean growth and yield under normal and different stresses conditions.

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