Chapter 7 Effects of selenium on yield, seed size, and phenolic compound content of common bean (*Phaseolus vulgaris* L.)

Capítulo 7 Efectos del selenio en el rendimiento, tamaño de la semilla y contenido de compuestos fenólicos del frijol común (*Phaseolus vulgaris* L.)

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Abstract

Beans are some of the most important legumes in human nutrition since they contain various secondary metabolites with antioxidant activity, such as phenolic compounds, associated with the color of the seed coat. Several reports indicate that beans with dark colors (black, red, brown, etc.) provide the highest contents of phenolic compounds, while those with light-colored seed coats have the lowest contents. Furthermore, selenium (Se) is an essential microelement for humans since it acts as an antioxidant and can help prevent various types of cancer and maintain good immune system functioning. This work aims to determine the effects of selenium on the yield, seed size, and phenolic compound content of common bean varieties with white seed coats. Four (0, 2.5, 5, and 10 µM) concentrations of sodium selenite (Na_2SeO_3) were evaluated during the cultivation of three beans (*Phaseolus vulgaris* L.) varieties with white coats, named OX-7, OX-11, and OX-14. Selenium concentrations were applied along with irrigation every 15 days. The OX-7 variety had the longest seeds, while the OX-11 and OX-14 varieties had the highest and lowest numbers of pods, respectively, and the highest and lowest yields. The highest content of phenolic compounds was obtained in the OX-11 variety, with the application of 5 µM Na_2SeO_3. Moreover, the highest concentration of flavonoids was found in OX-11, with both 5 and 10 µM Na_2SeO_3 treatments, as well as in OX-14 treated with 2.5 µM Na_2SeO_3. These findings indicate that the beneficial effect of selenium depends on the concentration, variety, and stage of plant development.

Secondary metabolites, Phenolic compounds, Legumes, Beneficial element

Resumen

El frijol es una de las leguminosas más importantes en la nutrición humana debido a que contiene varios metabolitos secundarios con actividad antioxidante, como los compuestos fenólicos, asociados con el color de la testa de la semilla. Algunos reportes indican que los frijoles de colores oscuros (negro, rojo, café, etc.) proporcionan un alto contenido de compuestos fenólicos, mientras que aquellas semillas con testa de colores claros tienen contenidos bajos. Por otro lado, el selenio (Se) es un microelemento esencial para los humanos debido a que actúa como un antioxidante, puede ayudar a prevenir varios tipos de cáncer y mantener el funcionamiento del sistema inmune. El objetivo de este trabajo es determinar los efectos del selenio en el rendimiento, el tamaño de la semilla y el contenido de compuestos fenólicos de variedades de frijol común de testa blanca. Se evaluaron cuatro (0, 2.5, 5 y 10 µM) concentraciones de selenito de sodio (Na_2SeO_3) durante el ciclo de cultivo de tres variedades de frijol (*Phaseolus vulgaris* L.) con testa de color blanca, codificados como OX-7, OX-11 y OX-14. Las concentraciones de selenio se aplicaron junto con el riego cada 15 días. La variedad OX-7 presentó las semillas más grandes; mientras que, las variedades OX-11 y OX-14 tuvieron el mayor y el menor número de vainas, respectivamente, así como los mayores y menores rendimientos. El mayor contenido de compuestos fenólicos se obtuvo en la variedad OX-11, con la aplicación de 5 µM Na_2SeO_3. También, la mayor concentración de flavonoides se encontró en la variedad OX-11, con ambos tratamientos de 5 y 10 µM Na_2SeO_3, al igual que OX-14 tratada con 2.5 µM Na_2SeO_3. Estos hallazgos indican que los efectos benéficos del selenio dependen de su concentración, la variedad y el estado de desarrollo de la planta.

Metabolitos secundarios, Compuestos fenólicos, Leguminosas, Elemento benéfico

7.1 Introduction

7.1.1 General characteristics of selenium

Selenium (Se) can be found in five allotropic forms, two of which are amorphous with the remaining three crystalline. This element can form molecules with a ring structure consisting of eight atoms and chain molecules of considerable length. Ring-shaped molecules are unstable and occur in red α β crystalline forms (Kieliszek, 2019).

Selenium is an essential microelement for humans and very important for cell metabolism, especially for antioxidant reactions (Woch & Hawrylak-Nowak, 2019). Its deficiency in the human body can lead to heart disease, viral infections, hyperthyroidism, diabetes, and cancer (Pannico *et al.*, 2020). This element enters the food chain mainly through the diet, and its level in food depends on the bioavailable reserves in the soil and the absorption and accumulation capacity of plants (Hajiboland *et al.*, 2015). Moreover, it is commonly added to the diet as sodium selenite (Na_2SeO_3). However, there is growing interest in dietary supplementation with organic selenium. Organic sources are more efficiently assimilated than inorganic selenium sources and are considered less toxic and, therefore, more appropriate for use as food supplements (Shini *et al.*, 2015).

The World Health Organization (WHO) recommends a daily selenium dose of 55 µg for adults. This required amount of Se changes with sex and age, 40-70 µg for men and 45-55 µg for women, while for children, the recommended daily dose is 25 µg (Kieliszek, 2019).

Selenium has been identified as a cofactor of the enzyme glutathione peroxidase, a catalyst in the reduction of peroxides that can damage cells and tissues, and can act as an antioxidant (Mezeyová *et al.*, 2020). Besides, Se is incorporated into selenoproteins that exert antioxidant and anti-inflammatory effects. The selenoprotein family in humans includes the following enzymes: glutathione peroxidases (GPX1-GPX4 and GPX6), thioredoxin reductase (TXNRD1-2), thioredoxin-glutathione reductase (TXNRD3), iodothyronine deiodinases (DIO1-3), selenophosphate (SEPHS2), and methionine sulfoxide reductase B1 (MSRB1) (Zoidis *et al.*, 2018). These selenoproteins depend mainly on Se intake through the diet. Selenoproteins are essential for human health, mainly due to their antioxidant activity (Mezeyová *et al.*, 2020).

Furthermore, selenomethionine is the main chemical form of selenium in plants, while selenocysteine predominates in animals. Therefore, selenoamino acids (selenocysteine and selenomethionine) are necessary for the synthesis of selenium-containing peptides and proteins (Shini *et al.*, 2015).

The effects of selenium have been addressed in many aspects of biomedicine, biochemistry, and environmental science. Selenium also has agricultural applications as a soil fertilizer and animal feed (Natasha *et al.*, 2018). The application of Se at low concentrations has emerged as a possible alternative to synthetic fungicides for controlling plant diseases and reducing their potentially dangerous effects on the environment and human health (López-Velázquez *et al.*, 2019)

7.1.2 Selenium in the soil

Selenium exists naturally in the Earth´s crust and is highly available to living things in arid areas with alkaline soils. Selenium concentrations are low, from 0.01 to 2.0 mg/kg, with an average of 0.4 mg/kg, although concentrations of 1200 mg/kg can be found in seleniferous soils (Becvort-Azcurra *et al.*, 2012). Selenium in the soil exists in four oxidation states: selenite (Se^{4+}), selenate (Se^{6+}), elemental selenium (Se^0), and selenide (Se^{2-}). Plants can absorb selenium in the forms of selenite and selenate, both of which are components of the most common inorganic compounds present in the soil (León-Morales *et al.*, 2019), but selenium can also be present in organic forms such as selenomethionine (SeMet), selenocysteine (SeCys), and methyl selenocysteine (MeSeCys) (Natasha *et al.*, 2018). Selenium can bind with organic and inorganic elements and can form complexes with different elements, such as hydrogen, oxygen, iron, and halogens (Natasha *et al.*, 2018).

Soil is the primary source of Se for plants, while plants are the main sources of Se for humans and animals (Shini *et al.*, 2015). Application of fertilizer containing Se is the best possible strategy for enriching soils deficient in this element. The use of organic fertilizers amended with Se is widespread in countries with Se-deficient soils (Natasha *et al.*, 2018).

7.1.3 Selenium uptake and metabolism in plants

Plants assimilate Se mainly in the form of Se^{6+} or Se^{4+}. Selenate is absorbed by the plasma membranes of root cells using sulfate (SO_4) assimilation pathways with the action of the enzyme sulfate permease, while selenite is absorbed through phosphate (PO_4) transporters (Pannico *et al.*, 2019).

The selectivity of these transporters depends on the plant species and is affected by the concentration of sulfate and the salinity, pH, and redox potential of the soil (Pannico *et al.*, 2020). Different types of sulfate carriers may have different selectivity for selenium and sulfur. However, compared to selenate, selenite is less soluble, more phytotoxic, and more difficult to transport and accumulate in plant tissues (Pannico *et al.*, 2019).

Selenate can access the SO_4 assimilation pathway and be reduced through Se^{4+} to Se^{2-} (Malagoli *et al.*, 2015) with greater incorporation of selenium into amino acids, especially in the accumulation of low molecular weight methylated species in plants (Wrobel *et al.*, 2020). Methylated Se species have shown biological activity of great relevance to human health. Moreover, a defensive mechanism found in accumulator and hyperaccumulator plants is based on the synthesis of various methylated selenium species that cannot be integrated into the protein structure (Wrobel *et al.*, 2020).

Some plants accumulate organic Se compounds such as methyl selenocysteine (MeSeCys), γ-glutamyl-MeSeCys, and selenocysteine (SeCys). Moreover, Se can also volatilize from plants in the form of dimethyl selenide or dimethyl diselenide, which are produced from SeMet and methyl SeCys, respectively (Malagoli *et al.*, 2015).

The phytotoxicity of Se in plants is mainly due to its incorporation into the amino acids selenocysteine and selenomethionine, which replace their sulfur analogs in plant proteins (Pannico *et al.*, 2020). Selenium uptake capacity depends mainly on plant species, with most agricultural and horticultural plants classified as nonaccumulators. At the same time, accumulators can grow in soils with high concentrations of salts (Mimmo *et al.*, 2017). Plants have been classified according to their Se accumulation capacity as hyperaccumulators (1000-15000 mg/kg), accumulators (do not exceed 1000 mg/kg), and nonaccumulators (<100 mg/kg) (Wrobel *et al.*, 2020).

Selenium accumulates mainly in the sprouts of plants. However, the degree of transfer of Se from the root to the aerial part of a plant depends on the plant species and the type and form of the Se species present in the soil (Natasha *et al.*, 2018). Selenate accumulates more readily in the aerial part of the plant, while most of the selenite remains in the roots and is quickly converted to organic forms (Natasha *et al.*, 2018).

7.1.4 The role of selenium in plant growth and physiology

Selenium, at low concentrations, acts as an antioxidant and can stimulate plant growth and improve tolerance to oxidative stress. In contrast, it acts as a pro-oxidant at high concentrations, which reduces plant growth by interfering with the sulfur metabolic pathway (Pannico *et al.*, 2019). A low concentration of Se stimulates antioxidant activity and the potential of the plant to cope with biotic or abiotic stress. On the other hand, high Se concentrations are tolerated by accumulator and hyperaccumulator plants, causing adverse effects in nonaccumulator plants (Wrobel *et al.*, 2020).

The growth of lettuce plants has been evaluated through the application of sodium selenite (Na_2SeO_3). The results showed that the optimal dose for plant growth was 7.35 µM (Nawaz *et al.*, 2014). Furthermore, selenium has been applied to tomato and pepper plants in the form of sodium selenite (Na_2SeO_3) in a 50% nutrient solution with concentrations of 5, 10, and 20 µM After 20 days, greater root and sprout growth was observed, and increases in dry biomass in both tomato and pepper were observed with the 5 µM concentration (Saldaña-Sánchez *et al.*, 2019).

Rice seedling growth has been evaluated with sodium selenate (Na_2SeO_4) applied at 15, 30, 45, 60, 75, 90, and 105 mg/kg. The results indicated that the highest growth occurred at a concentration of 15 mg/kg (Du *et al.*, 2019). Furthermore, the effect of foliar application of Se at concentrations of 5, 10, and 20 mg/L Na_2SeO_4 on grape plants (*Vitis vinifera* L.) has been evaluated. The results showed that the application of Se had a positive effect on the height of the plant, the number of leaves, and the leaf area, especially at a concentration of 5 mg/L (Karimi *et al.*, 2020).

To evaluate the germination and initial growth of pepper (*Capsicum annum* L.) and radish (*Raphanus sativus* L.), Na_2SeO_3 and Na_2SeO_4 were applied at concentrations of 1.25, 2.5, and 5 µM. In general, the germination percentage of pepper increased, but germination of radish remained unaffected. The addition of selenite increased the heights of the seedlings in both radish and pepper at a 5 µM concentration, while selenate at a 1.25 µM concentration improved length and number of roots in pepper and radish; height increased with this same concentration (León-Morales *et al.*, 2019).

7.1.5 The importance of beans

Mexico has been recognized as the primary center of bean diversification. This crop is considered one of the oldest; some of the archaeological finds in Mexico and South America indicate that it was known 5000 years BC (Quintana-Blanco *et al.*, 2016). In Mexico, beans are the legumes with the highest human consumption, ranging from 110 g to 10.38 kg per person in a year (SAGARPA, 2017). In Mexico, 70 species of the genus *Phaseolus* are reported out of the 150 species that exist in the world; of these, five have been domesticated: *Phaseolus vulgaris* (common bean), *P. coccineus* (ayocote bean), *P. lunatus* (lima bean), *P. acutifolius* (tepary bean), and *P. dumosus* (year-long bean) (Alcázar-Valle *et al.*, 2020).
Bean grains are sources of carbohydrates, proteins, lipids, B vitamins, fiber, minerals, and bioactive compounds with high antioxidant activity, such as flavonoids, anthocyanins, polyphenols, tannins, and flavones (García-Díaz *et al.*, 2018). Among the *Phaseolus vulgaris* varieties, there are different types of grains. Commercial value is influenced by characteristics such as size, color, grain uniformity, cooking time, and flavor (Mederos, 2006). Depending on the type of bean, the protein content varies from 14 to 33%, being rich in amino acids such as lysine and phenylalanine but with deficiencies in sulfur amino acids such as methionine and cysteine. Nevertheless, according to biological evaluations, the protein quality of cooked beans can reach as high as 70% (Ulloa *et al.*, 2011).

The color of the bean seed coat is often highly variable. This characteristic is determined by nine main genes that are responsible for generating changes in the patterns of seed color variation ranging from homogeneous primary colors or primary colors with secondary colors expressed as variegated spots, marks, stripes or patterns to combinations of two phenotypic expressions up to a uniform color (García-Díaz *et al.*, 2018). The color of the bean seed coat is related to the contents of various phenolic compounds, mainly tannins, flavonoids, and anthocyanins (Chávez-Mendoza *et al.*, 2019). Some authors, such as Xu & Chang (2009), have reported that black bean seeds provide a higher content of phenols, while white bean seeds have a lower content.

7.1.6 Phenolic compounds of beans

Phenolic compounds are molecules that contain one or more aromatic or benzene rings that are attached to one or more hydroxyl groups. These compounds are widely distributed in nature, especially in plants such as cereals, fruits, and vegetables, whether in stems, roots, flowers, fruits, or seeds. Therefore, they play various roles in metabolism, growth, reproduction, and protection against pathogenic organisms such as viruses, bacteria, and fungi (Abarca-Vargas & Petricevich, 2019). They contribute to some organoleptic properties of plant foods, for example, color and flavor.

Studies carried out by Xu & Chang (2009) indicate that the content of phenolic compounds in beans is higher than in other legumes, such as lentils, chickpeas, and soybeans. Phenolic compounds have a wide range of biological effects, with their anticarcinogenic and anti-inflammatory activities highlighted (Pérez-Pérez *et al.*, 2019).

Phenolic compounds are divided into hydroxycinnamic acids and hydroxybenzoic acids. The mean phenolic compounds in bean are ferulic, p-coumaric, caffeic, and sinapic acids, which are hydroxycinnamic acids. Gallic, p-hydroxybenzoic, vanillin and syringic acid are hydroxybenzoic acids (Yang *et al.*, 2018)

Flavonoids are low molecular weight compounds that can act as inhibitors of the growth of tumors and some types of cancer; together with phenolic acids and tannins, they increase the antioxidant capacity of foods due to their high redox potential (Pérez-Pérez *et al.*, 2019). However, various factors can degrade these compounds, such as storage at high temperatures and exposure to light. The main flavonoids present in bean seed coats are quercetin and kaempferol (Capistrán-Carabarin *et al.*, 2019).

Common bean is a food source rich in proteins, lipids, vitamins, minerals, bioactive molecules, and compounds with high antioxidant activity, such as phenolic compounds, which are associated with the color of the seed coat (Xu & Chang, 2009). Black bean seeds provide a higher content of phenolic compounds than white bean seeds. Phenolic compounds exert various biological effects, in particular, antioxidant, anticarcinogenic, and anti-inflammatory activities.

Selenium plays a vitally important role because it is an essential element for humans as a component of several enzymes, such as glutathione peroxidase (GSH-Px), which protects the human body against oxidizing agents. Selenium also has anticancer and anti-inflammatory properties, so a deficiency in this element can alter the body´s physiological functions, causing heart disease, diabetes, and cancer (Pannico *et al*., 2020).

An option for increasing Se intake is to consume crops enriched or supplemented with this element. Biofortification is the process of increasing the bioavailable content of specific elements in the edible parts of plants through agricultural intervention or genetic selection. Biofortification with Se seeks to improve the nutritional quality of vegetables, which implies the production of crops with more significant health benefits. The level of Se in food depends on the bioavailable reserves in the soil and the absorption and accumulation capacities of plants.

This work aimed to determine the effect of selenium on the phenolic content of the common white bean seed. Various concentrations of sodium selenite (Na_2SeO_3) were evaluated through application to the roots with irrigation every 15 days during bean cultivation until seeds were obtained.

7.2 Methodology

7.2.1 Establishment of the experiment

Bean seeds were sown in germination trays with a commercial substrate (SUNSHINE, 90% sphagnum and 10% vermiculite, dolomite, limestone) under greenhouse conditions at an average temperature of 24 °C and average relative humidity of 52.6%. At 21 days after sowing, seedlings of uniform size were selected and transplanted into pots with the commercial substrate (SUNSHINE), with two plants per pot. Four concentrations of sodium selenite (Na_2SeO_3) were used for this experiment: 0, 2.5, 5, and 10 µM, as well as three bean varieties (OX-7, OX-11, and OX-14). Different concentrations of Na_2SeO_3 were applied to the roots together with irrigation every 15 days. There were six replicates (six pots) for each bean variety and each Se treatment, for a total of 120 pots.

7.2.2 Determination of the number and lengths of pods

Four months after the establishment of the experiment, pods were collected. The lengths of 20 pods were measured for each combination of bean variety and treatment. A total of 240 pods were used. An electronic Vernier caliper was used to measure pod length.

7.2.3 Determination of bean seed size

The length, width, and thickness of each seed obtained were determined. Measurements were made on 20 seeds per replicate for each bean variety and for each treatment for a total of 1440 seeds. An electronic Vernier caliper was used to measure the seeds.

7.2.4 Determination of the weight of 100 seeds

For seed weight, 100 seeds were counted for each treatment for varieties OX-7 and OX-11. For variety OX-14, the weight of 50 seeds was recorded.

7.2.5 Preparation of extracts for the determination of phenolic compounds

Extracts were prepared from white bean seeds. First, each sample of seeds was ground in a coffee grinder until a very fine powder was obtained. Next, we weighed 1 g of fine powder and added 10 mL of a solution composed of acetone, water, and acetic acid in a 70:29.5:0.5 (v/v/v) ratio (Gu *et al.*, 2002). Then, maceration was carried out at room temperature in the dark with stirring for 17 h. After this time, the extracts were centrifuged at 4000 rpm for 8 min, washed, and centrifuged again under the same conditions. Subsequently, the supernatants from the maceration and washing were combined for each sample. Finally, the extracts were concentrated at 40 °C in a rotary evaporator (Buchi, R-100, Switzerland). The extracts were stored at -20 °C until analysis (Alcázar-Valle *et al.*, 2020).

7.2.6 Determination of total phenolic content

Total phenolic content was determined using the Folin-Ciocalteu (F-C) method. The previously obtained extracts were used; 50 µL of each sample was mixed with 3 mL of distilled water, 250 µL of the F-C reagent (2N), and 750 µL of the sodium carbonate solution (7%, w/v) and incubated at room temperature for 8 min. Next, 950 µL of distilled water was added to each sample, and the solutions were mixed and left in darkness for 2 h to read the absorbance at 765 nm with a spectrophotometer (Tecan, Infinite M200 Pro, Switzerland). Phenolic compounds were quantified using a gallic acid standard curve (0 to 500 mg L^{-1}) and reported in gallic acid equivalents (GAE) per gram of sample (Alcázar-Valle *et al.*, 2020).

7.2.7 Determination of flavonoid content

Flavonoid content was determined using aluminum chloride ($AlCl_3$) solution. First, 133 mg of $AlCl_3$ and 400 mg of sodium acetate ($C_2H_3NaO_2$) were weighed and dissolved in 100 mL of diluent solution (methanol-water-acetic acid; 140:50:10 v/v). Then, fifty microliters of each sample, previously extracted, was mixed with 700 µL of deionized water, and 250 µL of $AlCl_3$ solution was added. The resulting solutions were mixed and kept at room temperature for 30 min to complete the reactions. Finally, absorbance values were read at 410 nm with a spectrophotometer (Tecan, Infinite M200 Pro, Switzerland). Flavonoid quantification was performed using a quercetin standard curve (0 to 80 µg mL^{-1}) and expressed as quercetin equivalents (QE) per gram sample (Alcázar-Valle *et al.*, 2020).

7.2.8 Statistical analysis

An analysis of variance and comparison of means was performed with the Duncan multiple range test ($p \leq 0.05$) using the procedures of the SAS 9.1 statistical package.

7.3 Results

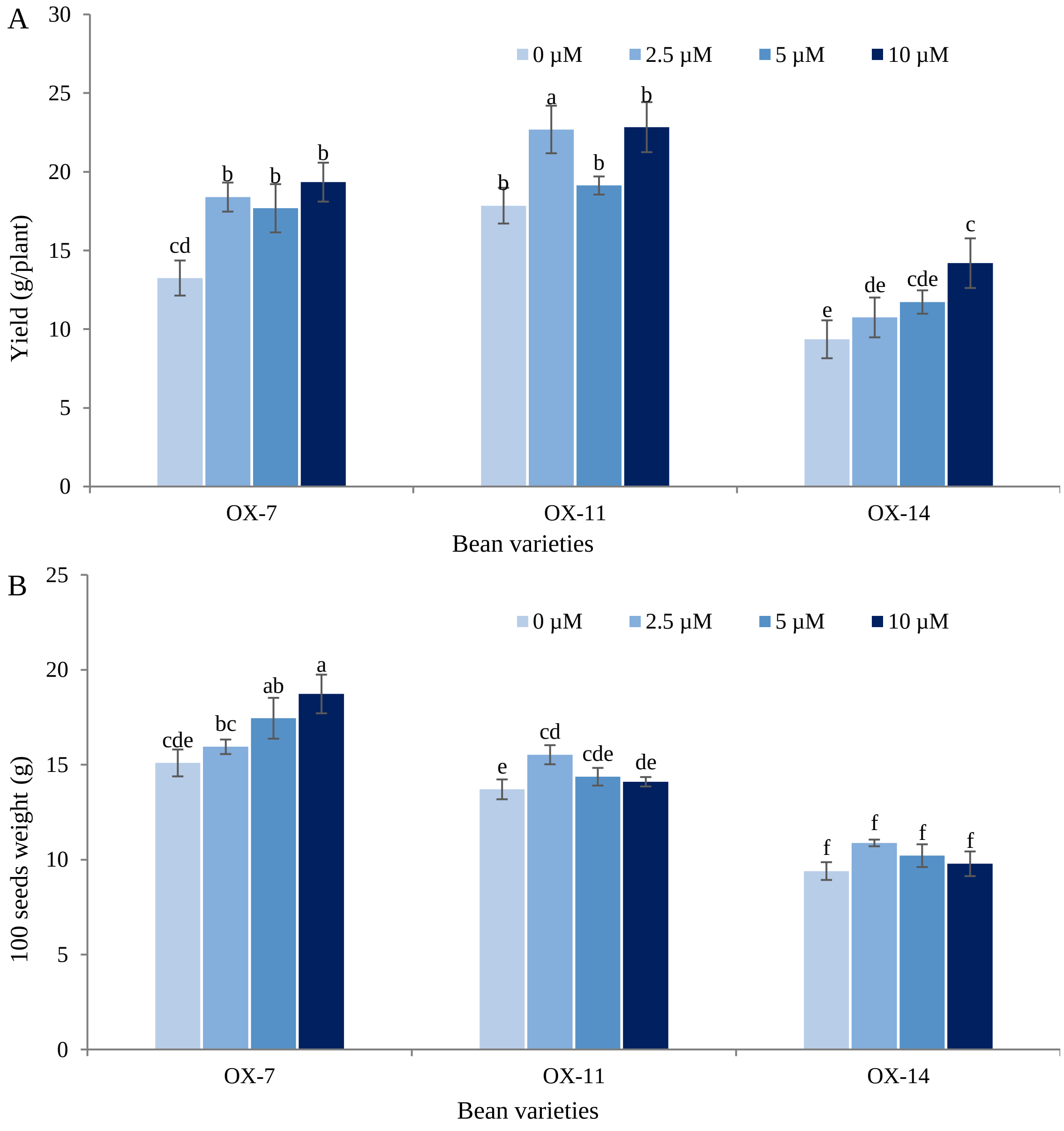
To evaluate the effect of sodium selenite (Na_2SeO_3), three bean varieties (OX-7, OX-11, and OX-14) were used, applying four concentrations of Na_2SeO_3 (0, 2.5, 5, or 10 µM). The treatments were applied by root application during the bean crop cycle. At the end, yield production, number, length of pods, and seed dimensions were obtained. Besides, the contents of total phenolic compounds and flavonoids in bean seeds were also determined.

7.3.1 Effect of selenium on the yield production of common bean

The OX-7 variety did not show significant variation in bean yield per plant among the 2.5, 5, and 10 µM Se concentrations; however, yields from these treatments differed significantly from that of the control (0 µM). In the OX-11 variety, no significant difference was observed between the 0 µM and 5 µM concentrations, but there were significant differences between the control (0 µM) and the 2.5 and 10 µM concentrations of Se. The OX-14 variety showed significant differences between the 0 and 10 µM concentrations, with a yield higher for the higher concentration of sodium selenite (Na_2SeO_3). The OX-11 variety had the highest yield, and OX-14 had the lowest yield (Graphic 7.1 A).

About the weight of 100 seeds for the OX-7 and OX-11 varieties, the OX-7 variety showed significant differences between the control (0 µM) and the 5 and 10 µM concentrations of Se, with a tendency for seed weight to increase as the concentration of sodium selenite (Na_2SeO_3) improved. In the OX-11 variety, a significant difference was observed between the control (0 µM) and the 2.5 µM Se concentration. In the OX-14 variety, the weight of 50 seeds was recorded because 100 seeds were not obtained for each replicate and treatment. Variety OX-14 did not show significant differences among the treatments applied (Graphic 7.1B).

Graphic 7.1 Total yield per plant (A) and 100-seed weight (B) of white bean varieties grown under the application of different concentrations of sodium selenite (Na_2SeO_3). Means with different letters indicate significant differences according to Duncan's test ($p<0.05$), ± standard deviation



7.3.2 Characteristics of bean seeds due to the effect of selenium

In the OX-7 variety, the greatest seed length was observed with the 10 µM concentration, while in this same variety, there were significant differences between the 0 and 10 µM concentrations in seed width and thickness. Variety OX-11 did not show significant differences in seed length between the 0 and 10 µM concentrations, while significant differences were observed in seed width and thickness for the 2.5 µM concentration. In the OX-14 variety, no significant differences were observed in seed length or thickness between the 0 and 10 µM concentrations. In contrast, there was a significant difference in seed width between the same concentrations. In general, variety OX-7 had the longest seeds, while variety OX-14 had the widest and thickest seeds (Table 7.1).

In the OX-7 variety, a significant difference was observed between the 0 and 2.5 µM Se concentrations. Varieties OX-11 and OX-14 had the highest and lowest number of pods, respectively, with the 2.5 µM Se concentration, although there were no significant differences with respect to the control. In general, the OX-11 variety exhibited the highest number of pods among the varieties; OX-7 had an intermediate number, while OX-14 had the lowest number of pods (Table 7.1). Regarding pod length, the most significant differences in length were observed in the OX-7 variety with the 2.5 and 10 µM Se concentrations. In comparison, in the OX-14 variety, the greatest length occurred with the 2.5 µM Se concentration. In the OX-11 variety, there were significant differences between the control (0 µM) and the 10 µM Se concentration (Table 7.1).

Table 7.1 Seed and pod characteristics of white bean varieties supplemented with different concentrations of Na_2SeO_3 (Se)

Bean varieties	Se [µm]	Seed lenght (mm)	Seed width (mm)	Seed thickness (mm)	Pod number	Pod length (cm)
OX-7	0	9.61±0.33ab	5.64±0.23c	4.51±0.26d	22.67±2.02ef	7.51±0.45bcd
	2.5	9.30±0.29de	5.63±0.16c	4.74±0.18c	28.33±2.07cd	8.02±0.39ab
	5	9.06±0.35f	5.70±0.20c	4.87±0.24bc	23.00±3.22ef	6.87±0.30e
	10	9.77±0.42a	5.90±0.19b	4.86±0.19bc	26.17±1.53de	8.22±0.19a
OX-11	0	9.62±0.31ab	4.92±0.14e	3.96±0.19e	32.67±2.80abc	7.73±0.22abc
	2.5	9.30±0.37cde	5.32±0.31d	4.49±0.37d	36.33±1.91a	7.35±0.36cde
	5	9.50±0.34bc	4.95±0.14e	4.02±0.16e	30.67±0.61bc	7.50±0.18cd
	10	9.62±0.34ab	4.85±0.14e	3.97±0.16e	33.33±1.37ab	7.41±0.15de
OX-14	0	9.52±0.46bc	6.14±0.19a	5.22±0.19^{a}	17.00±0.63g	6.89±0.34e
	2.5	9.11±0.68ef	5.73±0.39c	4.85±0.37bc	15.33±1.21g	7.76±0.27abc
	5	9.23±0.42def	5.91±0.20b	4.96±0.22b	19.33±0.93gf	7.46±0.28cd
	10	9.39±0.42bcd	5.99±0.20b	5.12±0.23^{a}	19.83±1.83gf	7.16±0.21de
Means with different letters indicate significant differences according to Duncan's test ($p<0.05$), ± standard deviation						

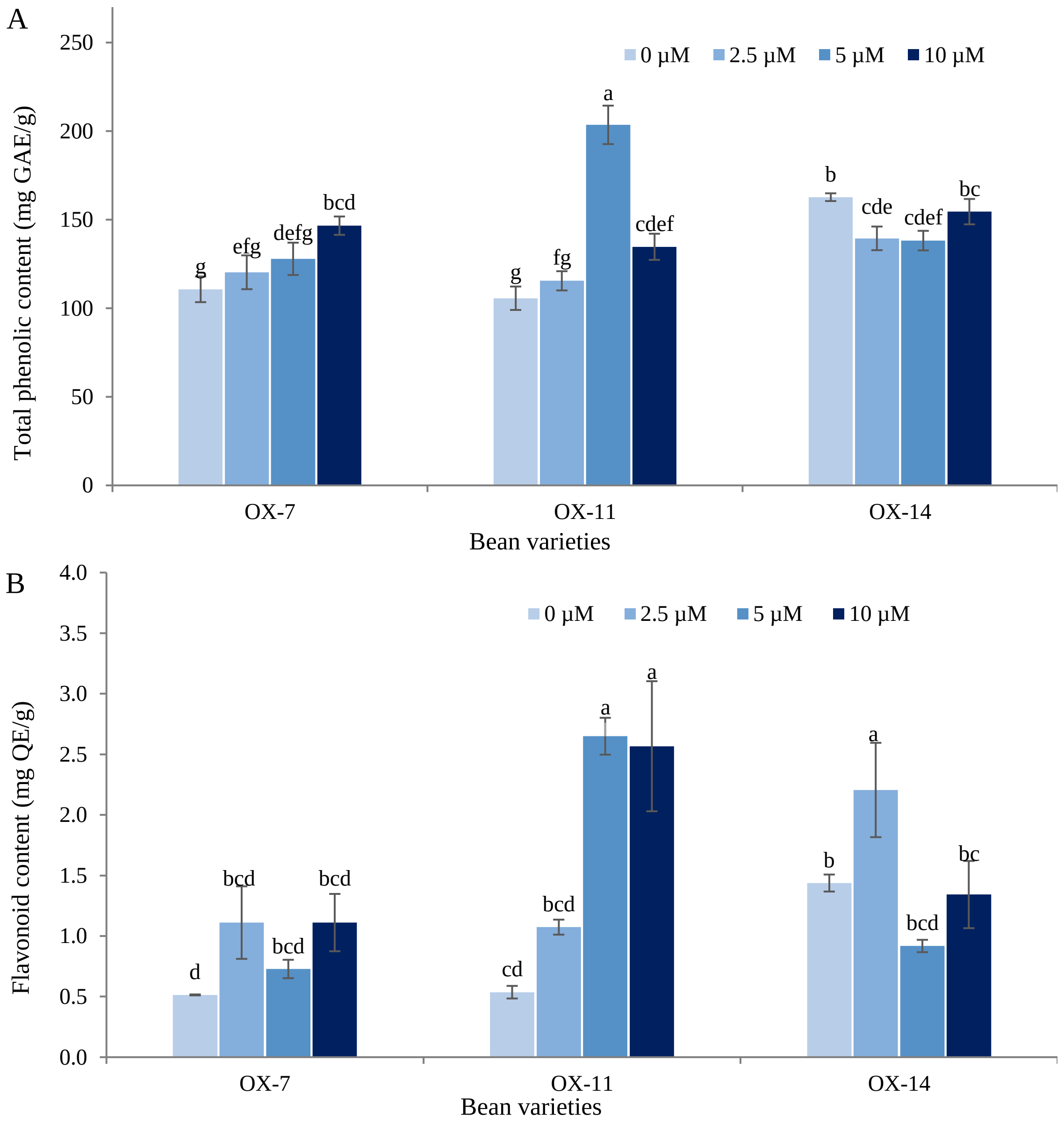
Source: Author's elaboration

7.3.3 Total phenol and flavonoid contents in bean seeds with different concentrations of Se

Concerning total phenols, the OX-7 variety showed a significant difference between the 0 and 10 µM concentrations, with a tendency for phenolic content to increase with increasing concentration of Se. Variety OX-11 showed significant differences for the 5 µM concentration of Se and the highest content of total phenols with this same concentration. In the OX-14 variety, no significant differences were observed among the applied treatments (Graphic 7.2A).

The content of flavonoids in bean seeds of variety OX-7 did not show significant differences among the applied treatments. The OX-11 variety showed significant differences at 5 and 10 µM Se concentrations. The variety OX-14 showed significant differences for the 2.5 µM Se concentration. In general, the highest flavonoid content was observed in the OX-11 variety at 5 µM, while at this same concentration, the OX-14 variety showed the lowest flavonoid content (Graphic 7.2B).

Graphic 7.2 Effect of Na_2SO_3 (Se) in the content of total phenolic compounds (A) and flavonoids (B) in seeds white bean varieties. Means with different letters indicate significant differences according to Duncan's test ($p<0.05$), ± standard deviation



7.4 Discussion

Biofortification with Se is an excellent strategy for increasing the Se content in food and improving the nutritional quality of vegetables, which implies the production of crops with more significant health benefits (León-Morales *et al*., 2019). The application of Se in a nutrient solution in soilless cropping systems (SCS) is the most efficient approach, as it can be applied more reliably through precise management of the composition and concentration of the nutrient solution (Pannico *et al*., 2020). Studies by Pannico *et al*. (2019) reported that biofortification with a 40 µM concentration of Na_2SeO_4 increased the Se content in red lettuce plants. In an investigation of wheat plants reported by Poplaciones *et al*. (2014), the greatest effect was found with the 10 µM concentration of Na_2SeO_4.

Seed size and growth habit are related to the efficiency of biomass allocation to grain in the growth environment. Nevertheless, these attributes depend on other seed characteristics, such as vigor (Morales-Santos *et al*., 2017). Vigor is a seed's biological potential for rapid and uniform establishment even under unfavorable plant conditions (Morales-Santos *et al*., 2017).

In this work, the OX-7 variety had the longest seeds, while the OX-14 variety had the thickest and widest seeds. According to studies reported by Alcázar-Valle *et al*. (2020), the species *P. coccineus* had the longest seeds. However, *P. vulgaris* was distinguished as the most heterogeneous species in terms of size differences among varieties.

The total yield per plant of white bean varieties OX-7, OX-11, and OX-14 varied among varieties and concentrations of Na_2SeO_3. This work reports the highest yield for the OX-11 variety, while the lowest yield is reported for the OX-14 variety. Studies by Premarathna *et al.* (2012) reported that the optimum dose in rice plants was 15 µM Na_2SeO_3. In this work, the highest yield was with a concentration of 10 µM Na_2SeO_3.

The highest flavonoid content was found in the OX-11 variety at a concentration of 5 µM, while the lowest content was found in the OX-14 variety at the same concentration. For the total phenolic content, a tendency was observed in the OX-7 variety: as the concentration of Se increased, the total phenolic content increased. Particularly in beans, in studies by Alvarado-López *et al.* (2019), the purple variety of the ayocote bean (*P. coccineus*) exhibited high concentrations of total phenolic compounds and flavonoids and high antioxidant activity. Xu & Chang (2009) mention the relationship between bean color and phenolic compound content, with light-colored beans containing lower levels of phenols than in dark-colored beans. However, in studies reported by Pérez-Pérez *et al.* (2019), Peruvian beans with yellow pigmentation showed higher content of phenolic compounds than did black beans.

In other crops, Malagoli *et al.* (2015) reported that a concentration of 2 µM Na_2SeO_4 was the best treatment for increasing the flavonoid content in tomato. Chomchan *et al.* (2017) reported an increase in phenolic compounds with 10 and 20 µM Na_2SeO_3 concentrations in rice. Studies by Woch & Hamrylak-Nowak (2019) showed that the optimal dose of Na_2SeO_3 was 20 µM in alfalfa, while Pannico *et al.* (2020) reported that the concentrations of 16 µM in cilantro and 8 µM in green and purple basil were the most effective for biofortification with Se and for increasing the contents of bioactive compounds.

Several authors, such as Schiavon *et al.* (2013), have reported the effect of selenium on the content of phenolic compounds in tomato leaves, where the highest contents of phenolic compounds were obtained at concentrations of 5 and 10 µM, studies conducted by Ghasemi *et al.* (2015) with garlic plants reported that the highest contents of flavonoids and total phenols were obtained with 10 µM concentration of Na_2SeO_4. In this study, the highest phenolic compound content was obtained in the OX-11 variety with 5 µM Na_2SeO_3, while the highest flavonoid concentration was found in the OX-11 (5 and 10 µM Na_2SeO_3) and OX-14 (2.5 µM Na_2SeO_3) varieties. Thus, variation is observed concerning the concentrations and sources of selenium to obtain the highest content of phenolic compounds in the various cultivars.

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7.7 Conclusion

Variety OX-7 had the longest seeds, and no significant differences in yield were observed among the applied treatments. Variety OX-11 had the highest number of pods, with no significant differences in pod length but with the highest yield. Variety OX-14 had the lowest number of pods and the lowest yield. Regarding flavonoid and total phenolic contents, variety OX-11 exhibited the highest phenol and flavonoid contents, while variety OX-12 had the lowest flavonoid content. Therefore, the beneficial effect of Se depends on the concentration and the variety and stage of development of the plant.

Future studies will consider including another source of selenium, such as sodium selenate (Na_2SeO_4), and other forms of selenium application, with foliar application being an alternative, and will determine the contents of inorganic and organic Se in bean seeds.

7.8 References

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