SILICON IS AN INHIBITOR OF CERTAIN ENZYMES *IN VITRO*

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Abstract. Silicon is considered an inorganic biostimulant and a prophylactic extracellular agent that allows the stimulation of a wide range of natural defences against abiotic and biotic stresses. However, little or no work has focused on the direct action of silicon on some enzymes. Indeed, during this study, the action of silicon was studied *in vitro* by direct contact of this element at different doses with the enzymatic extracts of *Trigonella foenum-graecum* L. (fenugreek) seeds. Our results showed that silicon strongly inhibited antioxidant and hydrolytic enzymatic activities. The percentage of this inhibition depends on the dose of silicon and the type of enzyme. The most sensitive enzymes to this inhibition were SOD and lipases whose activity was totally inhibited at 4 mM and 7 mM respectively. However, we report that the inhibitory action of silicon was limited to 50% for GPOX whatever the concentration of silicon used, the plateau being reached at 10 mM for GPOX and at 70 mM for proteases. Since these enzymes are mainly metallo-dependent, we hypothesize that their inhibition by silicon may be due to interactions between silicon and the metals involved in the functioning of each enzyme. Our study shows that silicon can be used as an inhibitor of enzymes involved in certain diseases.

Key words: antioxidants; hydrolases; inhibition; *in vitro*; seeds; silicon.

INTRODUCTION

Silicon (Si) is the second most abundant chemical element in the Earth's crust after oxygen. Silicon is never found in its free or pure state in nature, but only in combinations because it reacts rapidly with water and oxygen. It is often associated with oxygen with much greater strength than with other elements. It has an infinite preference for oxygen. Indeed, all the silicon found on earth is saturated with oxygen and is in the form of silica (SiO_2). Silica is the mineral form of silicon and it exists in many forms in nature (crystalline or amorphous or combined with other oxides). This form is found in certain rocks such as quartz, sand, amethyst or opal [78]. Silicon in mineral form is characterized by a crystalline structure, it is insoluble in water, and is therefore not assimilated by the living organism. While the plant feeds on this mineral by transforming it into organic silicon. Equipped with one or more carbon atoms that make it soluble in water, it is then assimilated by the body in the form of orthosilicic acid ($Si(OH)_4$). Plants rich in silica are nettle, horsetail, bamboo, diatoms (micro-algae from the seabed). These plants are widely used in herbal medicine and considered to be excellent natural remineralizers thanks to their richness in recognizable and assimilable silicon [39, 78]. However, silicon is not classified as an essential nutrient for plants and is considered to have little or no effect on plant metabolism in nonstressful situations [51]. However, several studies have shown the direct impact of Si on more fundamental metabolic processes, allowing improved growth and development under favourable conditions [25, 28]. However, silicon is best known for its beneficial effects on a wide range of plant species under environmental constraints [21, 28]. Hence, silicon is considered an inorganic biostimulant [70]. Indeed, over the past two decades, numerous laboratory, greenhouse and field experiments have demonstrated that Si is able to mitigate the biotic stress caused by plant diseases and pest attacks, as well as abiotic stresses such as drought, cold, high temperature, UV, salinity, nutrient deficiencies and metal toxicity [27]. Therefore, the ability of Si to attenuate stress plays a fundamental role in the life processes of plants [25, 28]. Si might be an important factor in plant functions, but little is known about silicon action in nonaccumulating plants or in plants in wild ecosystems [40].

Si uptake by plants requires a cascade of mechanisms. It is highly dependent on the soil type, source and amount of Si [21] and even the plant species [39]. However, while many studies have investigated the impact of Si on whole plants [21, 28], very few studies have focused on seed priming with Si [27, 30, 46]. This type of treatment may improve germination performance and tolerance to water stress [30] as well as salt stress [27], cadmium [65] and alkaline stress [1].

Moreover, sveral authors reported that the application of Si had an effect on the activities of enzymes, mainly antioxidant enzymes [44]. To this end, several authors have shown an increase in the activity of superoxide dismutase, peroxidases, phenol oxidase, glutathione reductase, phenylalanine ammonia lyase (PAL), ureases, acid phosphatases, nitrate reductase (NR) and glutamine synthetase under biotic and abiotic stress conditions in several plant species, including wheat, barley, cucumber and tomato [41]. However, in the case of silicosis, Kind *el al.* (1954) [45] observed slight inhibition of pepsin, trypsin, amylase, acetylcholinesterase, phosphatases, ureases and esterases *in vivo*. Dmitrenko *et al.* [26] also noted the inhibition of phosphorylase and proteinase. Similarly, Umemura *et al.* [77] reported that both ionic silicate and colloidal silica significantly inhibited the acid phosphatase activity of potato tubers and rice

leaves but not the acid phosphatase activity of sweet potato root and yeast hesokinase.

Thus, practically no other work has shown an inhibitory effect of silicon on plant enzymes in general or *in vitro* in particular.

The question that then arises is how Si acts on these enzymes. Is it a direct action that would inhibit this activity or indirect through other mechanisms? The aim of this work was to try to answer these questions through the study of the action of Si *in vitro* on several seed enzymes of *Trigonella foenum-graecum* L. (fenugreek). This action was studied *in vitro* via direct contact of this element at different doses with the enzymatic extracts from the seeds of *Trigonella foenum-graecum* L. We chose to work on soaked seeds with strong hydrolytic and antioxidant activities. The first group of enzymes concerns the enzymes involved in the hydrolysis of seed residues such as amylases (breakdown of starch into soluble sugars), lipase (hydrolyzes ester bonds of triglycerides) and proteases (breakdown of proteins into amino acids) and. Antioxidants are the second group of enzymes studied in the work, focusing on superoxide dismutase (dismutation of superoxide anion into hydrogen peroxide), catalase, ascorbate peroxidase and guaiacol peroxidase (elimination of hydrogen peroxide). To our knowledge, this study has never been carried out before.

MATERIALS AND METHODS

Plant material

Trigonella foenum-graecum (L), also known as fenugreek, is a legume plant well known for its therapeutic and nutritional properties. *Trigonella foenum-greacum* L. seeds are from a local variety (Halba) from eastern Algeria. Seeds were selected for their homogenous size prior to disinfection by bleaching.

Experimental protocol

The seeds were soaked for six hours in distilled water to facilitate grinding and activate the enzymatic activities studied. Afterward, 100 mg of fresh seed tissue was ground in a refrigerated (on ice) mortar using a pestle in 1.5 mL of 0.1 M Tris-HCl buffer, pH 8.1. Centrifugation was then carried out at 12000 rpm for 20 min at 4°C. For SOD, Cat and APX, the activities were measured on the same extracts of total proteins. These proteins were measured using the Bradford method (1976) [15] in order to calculate the enzymatic activities.

Silicon, in the form of sodium silicate (Na_2SiO_3, Sigma-Aldrich), was used because it is much more soluble than SiO_2. In addition, in *in vitro* experiments, SiO_2 poses a problem for reading the absorbance given the cloudiness of the solution.

For this *in vitro* study, the enzymatic extract of soaked seeds was mixed with a sodium silicate (Na_2SiO_3) solution (1:1 ratio, v/v) from 0.025 mM to 100 mM. This mixture was used for the measurement of the enzymatic activities studied as described below. To account for the high pH of the silicate solution, the pH of the reaction medium was adjusted prior to any measurement of enzymatic activity. The concentrations of the sodium silicate solutions shown on the graphs were calculated relative to the total volume of the reaction medium. The quantity of silicon alone is only a quarter of the amount of silicate compound (Na_2SiO_3).

Antioxydant enzyme activity assays

Measurements of superoxide dismutase (SOD), catalase (Cat), ascorbate peroxidase (APX), and guaiacol peroxidase (GPOX) activities were carried out *in vitro* on enzymatic extracts of fenugreek seeds.

Superoxide dismutase (SOD) (EC 1.15.1.1)

The SOD assay was carried out according to the method of Marklund and Marklund (1974) [54], slightly modified by Boucelha *et al.* [13]. The principle of the assay is based on the competition between the oxidation of pyrogallol by O_2^- and the disproportionation of O_2^- by SOD. The evaluation of the autooxidation of pyrogallol was carried out via differential measurements between a control and a test. The control cuvette contained 2.1 mL of 50 mM Tris-HCl buffer (pH 8.2) and 1 mM EDTA. The reaction was initiated by adding 100 µL of 1 mM pyrogallol (prepared in 0.01 N HCl). The increase in absorbance at 420 nm was due to the autooxidation of pyrogallol. The change in absorbance was measured every 30 seconds for four minutes. The test cuvette contained 2 mL of 50 mM Tris-HCl buffer at 50 mM pH 8.2, 1 mM EDTA and 100 µL of seed extract. The reaction was initiated by adding 100 µL of 1 mM pyrogallol. The change in absorbance was measured a second time. The % inhibition for one minute was calculated according to the following equation: [(ΔABScontrol - ΔABStest) / ΔABScontrol]×100.

SOD activity is expressed in units of SOD per minute and per milligram of protein.

Catalase (Cat) (EC 1.11.1.6)

CAT activity was determined by monitoring the decomposition of H_2O_2 at 240 nm ($\varepsilon = 36\ \mu M^{-1} \cdot cm^{-1}$) [6]. The reaction medium consisted of 2 mL of 100 mM potassium phosphate buffer (pH 7) and seed extract containing the enzyme (50 µL). The reaction was initiated by adding 10 mM H_2O_2. The activity was expressed in nanomoles of H_2O_2 degraded per minute and per milligram of protein.

Ascorbate peroxidase (APX) (EC 1.11.1.11)

Although the extraction was carried out without ascorbate, we can consider, at the level of the seeds, that it was the total APX activity since the APX chloroplasts are non-existent in these organs. Ascorbate peroxidase (APX) activity was measured according to the method of Nakano and Asada (1981) [60] by following the oxidation of ascorbate by hydrogen peroxide at a wavelength of 290 nm. The reaction medium consisted of 100 mM potassium phosphate buffer (pH 7) containing 1 mM Na_4EDTA and 10 mM H_2O_2. The reaction was initiated by adding

10 µL of ascorbate (50 mM). The enzyme activity was expressed as mmol of oxidized ascorbate·min^{-1}·mg^{-1} protein. This activity was calculated using the extinction coefficient of ascorbate, which is 2.8 mM^{-1}·cm^{-1}.

Guaiacol peroxidase (GPOX) (EC 1.11.1.7)

The activity of guaiacol peroxidase was determined according to the method of MacAdam *et al.* [53], with slight modifications to that of Boucelha *et al.* [13]. This technique is based on the increase in absorbance at 470 nm due to the polymerization of guaiacol to tetraguaiacol (oxidation), which results in an orange color in the presence of hydrogen peroxide. The extraction of the enzyme was carried out from 150 mg of cold-ground plant material in 2 mL of 0.1 M phosphate-potassium buffer pH 6.5 followed by centrifugation for 20 min at 12000 rpm at 4°C. The activity was measured on 100 µL of the enzymatic extract, to which 2 mL of the same phosphate buffer used for the extraction and 10 mM H_2O_2 were added. The reaction started as soon as 100 µL guaiacol was added to the reaction mixture. The activity was monitored as a function of time and was expressed in µmoles of oxidized guaiacol per minute and per mg of protein, using the molar extinction coefficient of tetraguaiacol (ε=26.6 mM^{-1}·cm^{-1}).

Hydrolase activities assays. Each enzyme has undergone a specific extraction.

Alpha-amylase (EC 3.2.1.1)

Alpha-amylase activity was estimated by the rate of starch hydrolysis. The residual starch content was measured according to the method of Shuster and Gifford (1962) [72] with slight modifications and was based is based on color development that results from iodine binding to starch polymers, forming a brown complex that absorbs at 620 nm. The extraction of the enzyme was carried out from 0.1 g of finely ground fresh plant material in the presence of 1 mL of acetate buffer (pH 5.6) at low temperature. The ground material was then centrifuged at 4°C for 15 minutes at 12000 rpm. The supernatant, which represented the extract, was retained for the amylase assay. For the measurement of α-amylase activity, 0.1 mL of enzymatic extract was added to 0.8 mL of 1% starch. The mixture was incubated at 37°C for 30 minutes. At the end of this period, the reaction was stopped immediately by the addition of 0.1 mL of 0.2 N sulfuric acid. The color reaction was carried out by adding 2.4 mL of iodine solution reagent to 0.4 mL of the incubated and nonincubated samples. The absorbance was read at 620 nm. A blank was made with 2.4 mL of iodine solution reagent and 0.4 mL of distilled water. The difference between the absorbances of the pure and incubated samples corresponded to the amount of degraded starch. The activity was expressed as mg of degraded starch per hour and per mg of protein.

Lipase (EC 3.1.1.3)

The activity of the lipase (triacylglycerol acylhydrolase) was determined by the method of Achakzai *et al.* [2] with slight modifications. This technique is based on colorimetric quantification of the nitrophenol released following the degradation of nitrophenyl-palmitate (substrate). The enzyme was extracted from 0.2 g of fresh plant material ground in 1 mL of 50 mM phosphate buffer, pH 8.0. After centrifugation for 20 min at 12000 rpm at 4°C, 3.75 mL of 48% ammonium sulfate was added to 1.5 mL of the supernatant, and the mixture was stirred for 30 min at 4°C. Then, the samples were centrifuged at 5000 rpm for 30 min. The supernatant was discarded, and the precipitate was dissolved in 1 mL of 50 mM phosphate buffer, pH 8.0. The solution obtained represented the enzymatic extract. The reaction mixture contained 0.1 mL of enzyme extract, 2.3 mL of 0.1 M Tris-HCl buffer (pH 9.0) and 0.6 mL of 4-nitrophenyl-palimitate. This substrate was obtained by adding 1 mL of the nitrophenol-palimitic acid complex, which was prepared at 20 mM in acetone, 8 mL of 0.1 M acetate buffer, pH 3.8 and 4 mL of 1% polyvinyl alcohol. The solution was adjusted to 20 mL with 0.1 M acetate buffer, pH 3.8. The samples were incubated for 1 h at 30°C, after which the absorbance was measured by spectrophotometry at 405 nm. Enzyme activity was calculated per mmol of p-nitrophenol released per hour and per mg protein. For this purpose, a standard curve of nitrophenol was produced.

Proteases (EC 3.4)

The protease activity was determined according to the method of McDonald and Chen (1965) [56]. This technique is based on a colorimetric assay of the tyrosine released following the degradation of casein (substrate). The extraction of the enzymes was carried out by grinding 150 mg of fresh plant material at low temperature in 1.5 mL of 50 mM Tris-HCl buffer at 50 mM pH 7.2 containing 0.01% ascorbic acid and 0.1% polyvinylpolypyrrolidone. After centrifugation at 4°C for 20 min at 12000 rpm, the supernatant (the enzyme extract) was recovered and kept cold until use. The reaction mixture contained 100 µL of enzymatic extract, 0.5 mL of Tris-HCl buffer (50 mM pH 7.2) and 0.5 mL of 2% casein dissolved in citrate phosphate buffer (50 mM pH 6.8). The mixture was incubated at 37°C for 1 h, and the reaction was stopped by crushing on ice and adding 1 mL of cold 10% TCA. Unhydrolysed casein substrate, if any, was removed by centrifugation at 5000 rpm for 5 min. An aliquot (0.5 mL) of the supernatant was taken, to which 2.5 mL of reagent containing 2.9% Na_2CO_3 and 0.3 N NaOH was added, followed by the addition of 0.75 mL of Folin Cioalteu's phenol reagent (Folin phenol: distilled water; 1:3). The samples were incubated at 37°C for 20 min, and the absorbance was read at 650 nm using a spectrophotometer. A standard curve constructed with tyrosine (1 mg·mL^{-1} in 0.2 M HCl) was used to calculate the protease activity. The activity was expressed as mg of tyrosine released per h^{-1}·mg^{-1} protein.

Variation percentage and statistical test

The percentage variation was calculated for each parameter studied according to the following relationship: Variation percentage = (Assay - Control) ×100 / Control.

The experiments were repeated at least five times. Student tests were carried out by Excel 2007 software.

RESULTS

Superoxide dismutase (SOD) activity

The results indicated that sodium silicate strongly inhibited SOD activity *in vitro*. We observed that the activity of SOD began to be inhibited at low concentrations of sodium silicate (5% inhibition at 0.2 mM) and reached 100% inhibition at 5 mM (Fig. 1).

Catalase activity (Cat)

The kinetics of catalase inhibition *in vitro* by sodium silicate were not linear. The enzyme began to be inhibited (6%) by 0.2 mM Na_2SiO_3 but in a slow manner, and it reached 100% inhibition at 15 mM (Fig. 1).

Ascorbate peroxidase (APX) activity

The *in vitro* results revealed that sodium silicate inhibited the enzymatic activity of APX in a linear manner with a strong correlation. This activity began to be inhibited (4%) by 0.5 mM Na_2SiO_3 and reached 100% inhibition at 25 mM (Fig. 1).

Guaiacol peroxidase (GPOX) activity

The *in vitro* results confirmed that sodium silicate inhibited lipase activity. The curve was not linear; the activity of the lipase began to be inhibited at low concentrations of sodium silicate (6% inhibition at 0.28 mM) and reached 100% inhibition at 7 mM (Fig. 1).

Amylase activity

The curve was linear with a strong correlation. The enzyme began to be inhibited very rapidly at very low concentrations (0.05 mM) and gradually reached 100% inhibition at 35 mM (Fig. 2).

Lipase activity

The *in vitro* results showed that sodium silicate inhibited lipase activity. The curve was not linear; the activity of the lipase began to be inhibited at low concentrations of sodium silicate (6% inhibition at 0.28 mM), reaching 100% inhibition at 7 mM (Fig. 2).

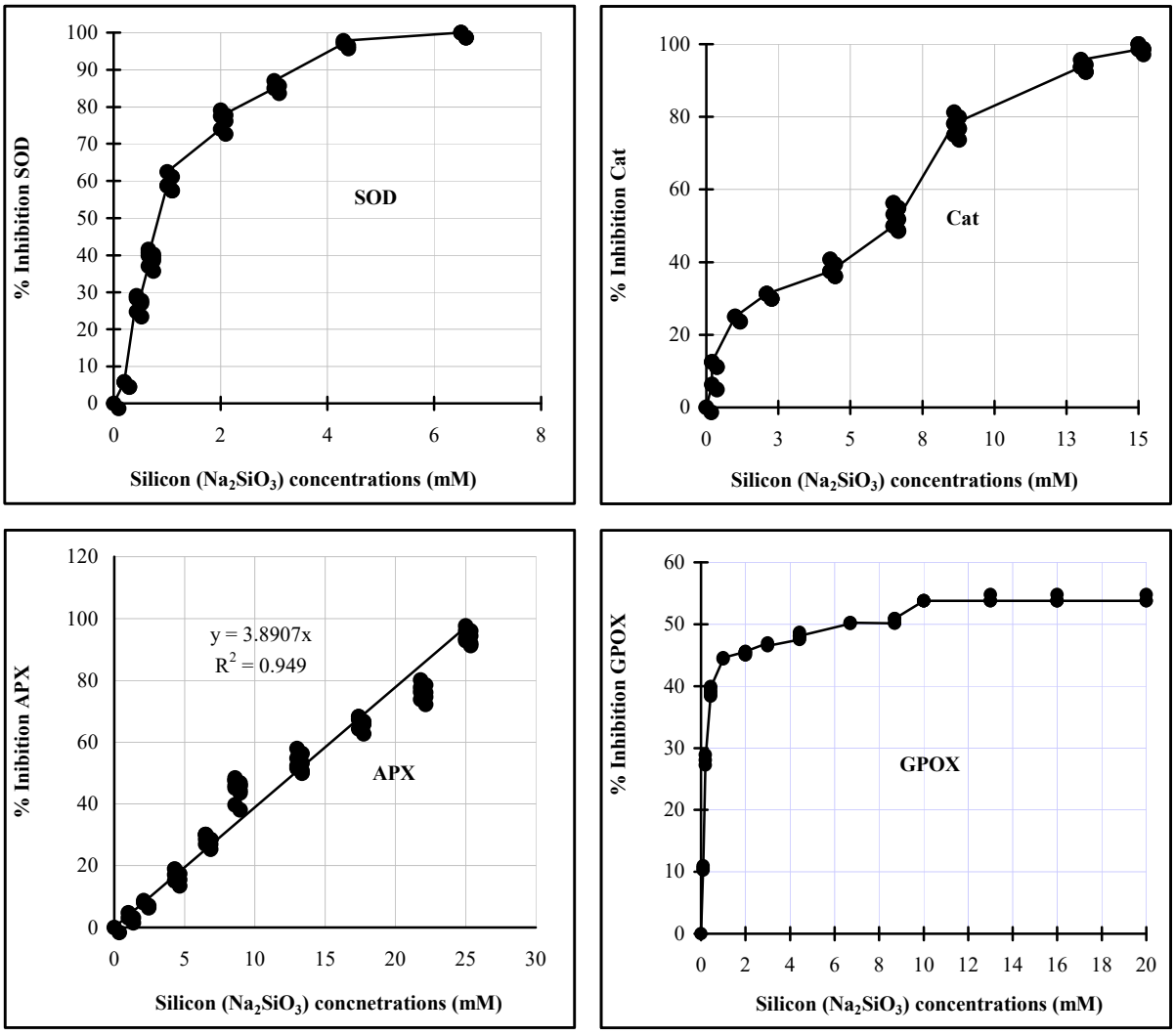

Figure 1. Curves representing the percentage of *in vitro* inhibition of the antioxidant activities of SOD, Cat, APX and GPOX in fenugreek seeds by different concentrations of sodium silicate.

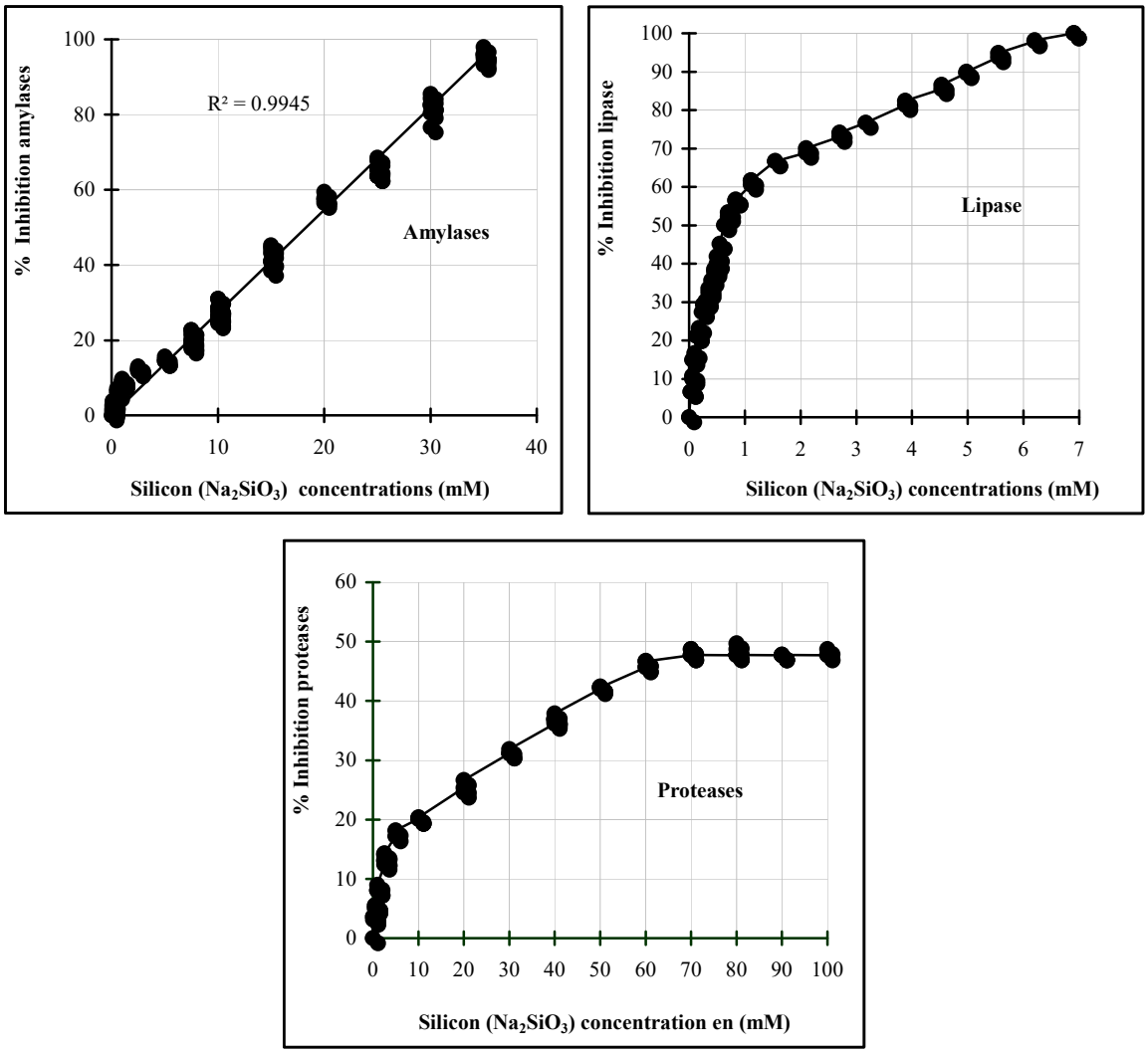

Figure 2. Curves representing the percentage of *in vitro* inhibition of the hydrolytic activities of amylase, lipases and proteases in fenugreek seeds by different concentrations of sodium silicate.

Protease activity

The results indicated that sodium silicate inhibited proteases *in vitro*. The kinetics were not linear. The enzyme began to be inhibited rapidly at very low concentrations (4% at 0.1 mM), but at 5 mM, the inhibition began to gradually slow, stopping at 48% of the maximum inhibition, reaching a plateau at 70 mM (Fig. 2).

DISCUSSION

We can assume that the quantities of sodium provided in the form of Na_2SiO_3 are not particularly toxic since they remain at physiological levels. Indeed, specialists agree on a maximum concentration of 30 mM in the cytosol [10, 74]. Furthermore, for all the enzymes tested in this study, inhibition begins at Na^+ values well below this threshold.

The objective of this *in vitro* study was to investigate the direct effect of silicon on enzymatic extracts of fenugreek seeds. The results showed that sodium silicate inhibited the enzymatic activities of antioxidants (SOD, CAT, APX and GPOX) and hydrolases (amylases, proteases, and lipases). However, the percentage of inhibition differed from one enzyme to another and depended on the concentration of the sodium silicate solution used. Since these enzymes are mainly metallo-dependent, we assume that their inhibition by silicon may be due to interactions between silicon and the other metals involved in the functioning of each enzyme. Moreover, several studies have shown that silicon reduces the toxicity caused by metal pollution in plants. However, the mechanisms of silicon action in plants are poorly understood [17, 57, 61, 62]. Several researchers accept that transition metals are essential to all forms of life. Iron, zinc, manganese, copper, and cobalt are essential trace elements in living beings [47]. Most of these metals are found in metalloproteins, which most often have an electron transfer function (ferredoxin, cupredoxins) or a catalysis function (metalloenzymes). In metalloproteins, metal ions can be coordinated directly with residues of the polypeptide chain or complexed by ligands that are themselves incorporated into the protein [37]. The definition of a catalytic site of an enzyme is a track that has attracted the attention

of many scientists in regard to understanding the function of an enzyme or designing an inhibitor molecule. The situation is more complex when the active site includes a metal center, and the catalytic process involves coordination between the arrival of a substrate and intramolecular transfers of electrons and protons [4].

In light of our results and existing data on silicon, we can assume that this redistribution of metals under the effect of silicon would disturb the chemical bonds that bind the enzymes and these metals, which play essential roles at the level of the active sites of these enzymes.

Superoxide dismutase (SOD)

Superoxide dismutases are nonheme metalloenzymes found throughout the living world, except in a few microorganisms [5]. Plants have three types of SODs containing prosthetic groups containing different metals: iron (Fe-SOD), manganese (Mn-SOD) or copper and zinc (Cu/Zn-SOD) [7, 14].

The strong inhibition of SOD by low concentrations of silicon could be due to the direct action of Si on the metal atoms of SOD (Fe, Mn, Zn and Cu). Indeed, studies on rice suggest that silicon application increases the ability of roots to oxidize iron, converting ferrous ions (Fe^{2+}) to ferric ions (Fe^{3+}) [64]. We speculate that this ability of silicon to translocate and oxidize iron could cause the destruction of its functional bond with SOD. Silicon increases the binding of manganese to cell walls, thus limiting its cytoplasmic concentration [48]. For this purpose, we hypothesize that stimulation of silicon to form strong bonds between manganese and cell walls would prevent the binding of Mn with SOD, since Mn-SODs are located mainly in the mitochondrial matrix [14, 23], in the matrix of glyoxisomes or bound to the peroxisome membrane of leaf cells [23].

On the other hand, Neumann and zur Nieden (2001) [61] suggested that intracellular silicon forms Zn-silicate complexes in the leaf cytoplasm, leading to less soluble zinc in cells. These silicates are then transformed into SiO_2 in the cytoplasm, while metals, particularly zinc and copper, are sequestered in the vacuole. This induces a decrease in the mobility of these metal ions in plants treated with silicon [29, 64], and Si significantly decreases the Zn content in several plant species, even in the presence of sufficient amounts of Zn in the soil. These authors concluded that the binding of Zn with Si in the roots prevents the translocation of Zn to the leaves. For copper, the deposition of silicon in plant cells causes the immobilization of copper ions, increasing the capacity of cell walls to bind with this metallic cation and contributing to the regulation of intracellular copper homeostasis [12, 64]. Additionally, silicon stimulates the synthesis of copper-binding molecules in roots and leaves, such as chelating proteins, amino acids and organic acids. Research has revealed that Si decreases the expression levels of two copper transporter genes in Arabidopsis roots [46].

On the basis of these observations, we can assume that the lack of availability of zinc and copper in the cytoplasm under the effect of Si would cause the inactivation of Cu/Zn-SOD. Thus, we explain the inhibition of this enzyme in the presence of Si by the binding of metal ions to the cell wall, whereas Cu/Zn-SODs are essentially found in free form in the cytosol [14], peroxisomes or apoplast [63].

Catalase (CAT)

Catalases are predominantly peroxisomal enzymes that catalyze the dismutation of hydrogen peroxide [7]. They are formed of four polypeptide chains, each comprising a heme group comprising an iron atom. These hemes and their protein environments are the active sites of the enzyme [49]. Moreover, studies have shown that the increase in the expression of Si transporters after its application can modulate the absorption and translocation of iron from the root to the plant by increasing the oxidative capacity of the roots, which transforms ferrous iron (Fe^{+2}) to ferric iron (Fe^{+3}) [64]. These characteristics can be explained by the influence of silicon on the regulation of the expression of genes involved in the strategy based on the reduction of iron acquisition, as well as genes involved in the biosynthesis of iron-chelating compounds (for example, organic acids and phenolic compounds) [62]. In this sense, several recent studies on rice [18] and cucumber [34] reported that the contribution of Si in the growth phase caused the formation of an iron deposit and a decrease in its absorption, supporting the hypothesis proposed by Coskun *et al.* [21] that silicon can cause apoplastic obstruction.

Chaiwong *et al.* [19] reported that there are strong signalling pathways between silicon and iron at the plant level; thus, we assume that the signalling between these two elements would cause biochemical and metabolic modifications at the cellular level. On the basis of these data, we propose that the inhibition of catalase by silicon is due to the capacity of the latter to oxidize and retranslocate the iron involved actively in the function of this enzyme. The oxidation or destabilization of ferric heme would induce the loss of its functional properties within the active site of catalase.

Additionally, Keilin and Hartree (1945) [42], Murali and Patel (2017) [59] reported that catalase inactivation requires oxygen and is greatly accelerated by the presence of metal ions such as cadmium, lead, zinc and mercury. This positive effect could thus be annihilated by silicon.

Peroxidases (APX and GPOX)

Plant peroxidases (POX) are a large multigenic family of heme enzymes whose primary function is to remove hydrogen peroxide (H_2O_2) generated during many reactions with O_2 [75]. There are three main classes of peroxidases whose molecular structures differ essentially at the ferric heme level [79]. Ascorbate peroxidase (APX), a class I peroxidase, is a metalloenzyme containing a ferriprotoporphyrin IX

group [66]. It is an intracellular, nonglycosylated peroxidase without a calcium or disulfide bridge [81]. Guaiacol peroxidase (GPOX) is a class of glycoproteins (class III peroxidase or secreted peroxidase) typical of green plants and belongs to a large family of multigenic proteins [58]. All class III peroxidases have a signal peptide at their N-terminal end [24].

A characteristic of extracellular peroxidases (class III) is the existence of two calcium binding sites, one in each conserved domain, that are essential for the proper functioning of the protein. Their absence notably decreases the activity of horseradish peroxidase (HRP) [32], and a change in the redox properties of the iron atom is then observed [71]. The absence of calcium atoms likely leads to major modifications in the structure of the active site. The active site of peroxidases is centered around heme, which is a protoporphyrin IX associated with a ferric iron atom [24]. Therefore, for the inhibition of these peroxidases by silicon, we propose the same explanation suggested in the case of catalase inactivation concerning ferric heme. Thus, we assume that the inhibition of peroxidases by silicon may also be due to a negative interaction (antagonism) between silicon and calcium involved in the functions of these enzymes. Indeed, studies have demonstrated the link between silicon accumulation and tissue calcium accumulation in several crop species [22, 52]. Silicon also acts on calcium by decreasing its activity, as shown by Bosnic *et al.* [12], in a Na^+ rich nutrient solution.

On the other hand, our results indicate that the inhibition of GPOX did not exceed 55% regardless of the concentration of sodium silicate used. To this end, we assume that the inability of silicon to completely inhibit the activity of GPOX is linked to the existence of several isoenzymes with different regulatory effects. These can be generated by posttranscriptional and posttranslational modifications [75]. GPOX signalling is certainly variable, but it seems that it can be refined by measuring isoforms [20].

Amylases

These enzymes are a relatively large class of hydrolytic enzymes called glycosidases. Alpha-amylases (α-1,4-glucan-4-glucanohydrolase, EC 3.2.1.1) are ubiquitous enzymes synthesized in all kinds of life [38]. Alpha-amylase can breakdown long-chain carbohydrates, such as starch amylose, into maltotriose and maltose or amylopectin into maltose, glucose and limited dextrin, whereas beta (β)-amylase is considered a major enzyme involved in seed germination and fruit ripening. β-amylase catalyzes the enzymatic degradation of the second α-1,4 glycosidic bond of nonreducing sugars, thereby removing maltose immediately. β-amylase breaks down starch into maltose [67].

All alpha-amylases bind to their active site a highly conserved Ca^{2+} ion, which is essential for structural integrity and for enzyme activity [3]. Calcium ions often play crucial roles in the structure, function and stability of α-amylase [76]. They are considered important for maintaining protein structures in their correct conformations [43]. The removal of calcium ions from barley α-amylase has been shown to irreversibly inactivate the enzyme, whereas bacterial α-amylase activity is restored after the addition of calcium ions [17]. Some reports indicate that the role of calcium ions in α-amylase is primarily structural, as their catalytic sites are distant from calcium binding sites [16].

In light of this knowledge, we can link the inhibition of amylases by silicon to the antagonism between this element and calcium, suggesting the same explanation suggested in the case of peroxidases. Additionally, Sarowar-Jahan *et al.* [69] reported that amylases are inhibited by heavy metal ions such as Ag^+, Hg^{2+}, Cu^{2+}, and Fe^{2+}. Similarly, silicon could have the same inhibitory effect on amylases as these heavy metals, but the mechanisms are not yet well defined.

Lipases

They belong to the subclass of hydrolases specific for carboxylic esters [68]. The lipase mechanism shows some similarities with that of active serine proteases, Beer *et al.* [9] indicating that the reaction is initiated by the attack of the carbon of the carboxylic function of the substrate by the hydroxyl group of the serine of the enzyme to form a first tetrahedral intermediate.

Thus, many studies have focused on lipase inhibitors and have provided information on their modes of action. Some of these inhibitors are capable of bonding covalently with catalytic serine, and this bond is possibly reversible in some cases [11]. Tetrahydrolipstatin (THL), a potent active site-directed lipase inhibitor, contains a β-lactone ring that is attacked by the catalytic serine of the active site of the lipase [50], resulting in the opening of this ring and the formation of a covalent bond with the enzyme, with the acylation of a hydroxyl group at the active site of the lipase. On the basis of these findings, we assume that the strong inhibition of lipase activity in fenugreek seeds is due to the ability of silicon to bind with serine, thereby inactivating the lipase, given that it was shown by Teschner *et al.* [73] that treatment with finely divided silicon dioxide allows the elimination of serine proteases from the plasma-derived protein composition.

Proteases

Proteolytic enzymes are ubiquitous hydrolases constituting a complex group of enzymes that differ in their properties, such as substrate specificity, active site and catalytic mechanism, optimum pH, temperature, and stability [8, 55]. Metalloproteases are constitutively present in all plant species [80]. They contain a metal ion at their active site, which acts as a catalyst on the peptide bonds of hydrolases [36]. The most widespread metal ion metalloproteases are zinc (Zn^{2+}) containing metalloproteases [33]. Other transition metals, such as Co^{2+} and Mn^{2+}, have been found at active sites, and some have been used to

restore the function of zinc metalloproteases, in which the Zn^{2+} nucleus has been chelated or removed [43].

Moreover, Hangauer *et al.* [31] reported that elimination of Zn^{2+} causes inactivation of metalloproteases. Indeed, the use of metal ion chelators such as EDTA allows the complete inhibition of these enzymes [35]. In light of these data and the results obtained in this study, we can link the inhibition of proteases by silicon to the capacity of the latter to interact with intracellular zinc, maintaining the same explanation suggested above, in the case of Zn-SOD.

On the other hand, our study revealed that the inhibition of proteases did not exceed 50% regardless of the concentration of sodium silicate used. We could explain this result by the fact that fenugreek seeds in the germination phase contain not only metalloproteases but also metal-independent proteases, particularly cysteine proteases. Moreover, several authors have shown that the proteases most involved in germination are cysteine proteases (CysProt) [55].

In conclusion, we assume that our *in vitro* study of the direct action of silicon at different doses on some antioxidant and hydrolytic activities of fenugreek seeds revealed that silicon seems strongly inhibits the activities of catalase, APX, GPOX, amylases, lipase and proteases, and the percentage of this inhibition depends on the concentration of silicon and types the enzyme. Our study showed that superoxide dismutase and lipases are the most sensitive enzymes to the inhibitory action of silicon, reaching 100% inhibition at low concentrations between 4 mM and 7 mM. While guaiacol peroxidase and proteases their inhibition remains stationary at 50% from 10 mM for GPOX and 70 mM for proteases.We hypothesize that this inhibition is linked to the interaction of silicon with the metals involved in the activities of these enzymes (Fig. 3). This discovery on the inhibition of enzymes by silicon can be used to treat certain diseases involving enzymes such as cancer and infectious and mental pathologies. This study represents only the beginning of long-term work aimed at better understanding the mechanisms involved in the inhibition of these enzymes by silicon. It would be necessary to carry out an in-depth study of the interaction of silicon with different enzymatic cofactors and to study the effects of this mineral on other enzymes, particularly phosphatases, ATP synthases and ATPases.

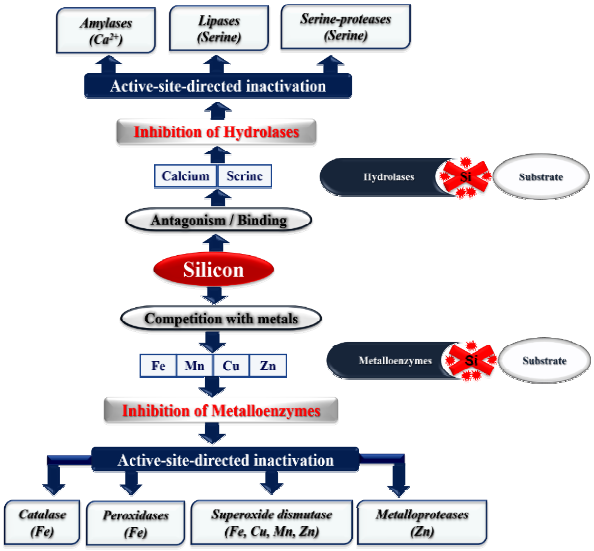

Figure 3. Model explaining the implication of silicon on the inhibition of enzymes *in vitro*. Silicon strongly inhibits the activities of catalase, APX, GPOX, amylases, lipase and proteases by interacting with metals involved in the activities of these enzymes. The result of this competition is the inactivation of their active site.

Abreviations. EC: The EC nomenclature (EC stands for Enzyme Commission numbers) is a numerical classification of enzymes, based on the chemical reaction they catalyze. As a nomenclature system for enzymes, each EC number is associated with a recommended name for the corresponding enzyme: **EC 1** = Oxidoreducatases; **EC 1.11** = Peroxidases; **EC 1.15** = Dismutase; **EC 3** = Hydrolases.

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REFERENCES

[1] Abdel Latef, A.A., Tran, L.S.P., (2016): Impacts of priming with silicon on the growth and tolerance of maize plants to alkaline stress. Frontiers in Plant Science, 7: 1-10.

[2] Achakzai, A.K.K., Kayani, S.A., Yaqoob, M., Nabi, A., (2003): Effect of fertilizer and inoculation on lipase and urease activity of mature soybean cv. Williams-82 seeds. Asian Journal of Plant Sciences, 2: 692-695.

[3] Aghajari, N., Feller, G., Gerday, C., Haser, R., (2002): Structural basis of α-amylase activation by chloride. Protein Science, 11: 1435-1441.

[4] Al-Attar, S., Rendon, J., Sidore, M., Duneau, J.P., Seduk, F., Biaso, F., Grimaldi, S., Guigliarelli, B., Magalon, A., (2021): Gating de l'accès au substrat et du transfert de protons à longue distance dans la nitrate réductase A d'*Escherichia coli*: le rôle essentiel d'un résidu de glutamate distant. ACS Catalysis, 11 (23): 14303-14318.

[5] Alscher, R.G., Erturk, N., Heath, L.S., (2002): Role of superoxide dismutases (SODs) in controlling oxidative stress in plants. Journal of Experimental Botany, 53: 1331-1341.

[6] Anderson, D., Prasad, K., Stewart, R., (1995): Changes in isozyme profiles of catalase, peroxidase and glutathione reductase during acclimation to chilling in mesocotyls of maize seedlings. Plant Physiology, 109: 1247-1257.

[7] Arora, A., Sairam, R.K., Srivastava, G.C., (2002): Oxidative stress and antioxidative system in plants. Current Science, 82: 1227-1238.

[8] Barrett, A.J., (1994): Proteolytic enzymes: serine and cysteine peptidases. San Diego: Academic Press, 244: 1-765.

[9] Beer, H.D., Wohlfahrt, G., McCarthy, J.E.G., Schomburg, D., Schmid, R.D., (1996): Analysis of the catalytic mechanism of a fungal lipase using computer-aided design and structural mutants. Protein Engineering, 9: 507-517.

[10] Benito, B., Haro, R., Amtmann, A., Cuin, T.A., Dreyer, I., (2014): The twins K^+ and Na^+ in plants. Journal of Plant Physiology, 171: 723-731.
[11] Borgström, B., (1988): Mode of action of tetrahydrolipstatin: A derivative of the naturally occurring lipase inhibitor lipstatin. Biochimica et Biophysica Acta (BBA)-Lipids and Lipid Metabolism, 962: 308-316.
[12] Bosnić, D., Bosnić, P., Nikolić, D., Nikolić, M., Samardžić, J., (2019): Silicon and iron differently alleviate copper toxicity in cucumber leaves. Plants, 8: 554.
[13] Boucelha, L., Djebbar, R., Abrous-Belbachir, O., (2019): *Vigna unguiculata* seed priming is 591 related to redox status of plumule, radicle and cotyledons. Functional Plant Biology, 46(6): 584-594.
[14] Bowler, C., Van Camp, W., Van Montagu, M., Inzé, D., Asada, K., (1994): Superoxide dismutase in plants. Critical Reviews in Plant Sciences, 13: 199-218.
[15] Bradford, M.M., (1976): A rapid and sensitive method for the quantitation of microgram quantities of protein utilising the principle of protein dye binding. Analytical Biochemistry, 72: 248-254.
[16] Buisson, G., Duee, E., Haser, R., Payan, F., (1987): Three-dimensional structure of porcine pancreatic alpha-amylase at 2.9 A resolution. Role of calcium in structure and activity. The EMBO Journal, 6: 3909-3916.
[17] Bush, D.S., Sticher, L., Van Huystee, R., Wagner, D., Jones, R.L., (1989): The calcium requirement for stability and enzymatic activity of two isoforms of barley aleurone α-amylase. Journal of Biological Chemistry, 264: 19392-19398.
[18] Carrasco-Gil, S., Rodríguez-Menéndez, S., Fernández, B., Pereiro, R., de la Fuente, V., Hernandez-Apaolaza, L., (2018): Silicon induced Fe deficiency affects Fe, Mn, Cu and Zn distribution in rice (*Oryza sativa* L.) growth in calcareous conditions. Plant Physiology and Biochemistry, 125: 153-163.
[19] Chaiwong, N., Bouain, N., Prom-U-Thai, C., Rouached, H., (2020): Interplay between silicon and iron signaling pathways to regulate silicon transporter Lsi1 expression in rice. Frontiers in Plant Science, 11: 1-9.
[20] Chaoui, A., Mazhoudi, S., Ghorbal, M.H., El Ferjani, E., (1997): Cadmium and zinc induction of lipid peroxidation and effects on antioxidant enzyme activities in bean (*Phaseolus vulgaris* L.). Plant Science, 127: 139-147.
[21] Coskun, D., Deshmukh, R., Sonah, H., Menzies, J.G., Reynolds, O., Ma, J.F., Kronzucker, H., Belanger, R.R., (2019): The controversies of silicon's role in plant Biology. New Phytologist, 221: 67-85.
[22] da Silva, D.L., de Mello Prado, R., Tenesaca, L.F.L., da Silva, J.L.F., Mattiuz, B.H., (2021): Silicon attenuates calcium deficiency by increasing ascorbic acid content, growth and quality of cabbage leaves. Scientific Reports, 11: 1770.
[23] del Río, LA., Sandalio, L.M., Altomare, D.A., Zilinskas, B.A., (2003): Mitochondrial and peroxisomal manganese superoxide dismutase: differential expression during leaf senescence. Journal of Experimental Botany, 54: 923-933.
[24] Delannoy, E., Marmey, P., Penel, C., Nicole, M., (2004): Les peroxydases végétales de classe III. Acta Botanica Gallica, 151: 353-380.
[25] Detmann, K.C., Araújo, W.L., Martins, S.C., Sanglard, L.M., Reis, J.V., Detmann, E., (2012): Silicon nutrition increases grain yield, which, in turn, exerts a feed-forward stimulation of photosynthetic rates *via* enhanced mesophyll conductance and alters primary metabolism in rice. New Phytologist, 196: 752-762.
[26] Dmitrenko, M.T., (1957): Vrachebnoe Delo, 6: 629.
[27] Ellouzi, H., Rabhi, M., Khedher, S., Debez, A., Abdelly, C., Zorrig, W., (2023): Silicon seed priming enhances salt tolerance of barley seedlings through early ros detoxification and stimulation of antioxidant defence. Silicon, 15: 37-60.
[28] Frew, A., Weston, L.A., Reynolds, O.L., Gurr, G.M., (2018): The role of silicon in plant biology: a paradigm shift in research approach. Annals of Botany, 121: 1265-1273.
[29] Greger, M., Landberg, T., Vaculík, M., (2018): Silicon influences soil availability and accumulation of mineral nutrients in various plant species. Plants, 7: 41.
[30] Hameed, A., Farooq, T., Hameed, A., Sheikh, M.A., (2021): Silicon-mediated priming induces acclimation to mild water-deficit stress by altering physio-biochemical attributes in wheat plants. Frontiers in Plant Science, 12: 625541.
[31] Hangauer, D.G., Monzingo, A.F., Matthews, B.W., (1984): An interactive computer graphics study of thermolysin-catalyzed peptide cleavage and inhibition by N-carboxymethyl dipeptides. Biochemistry, 23(24): 5730-5741.
[32] Haschke, R.H., Friedhoff, J.M., (1978): Calcium-related properties of horseradish peroxidase. Biochemical and Biophysical Research Communications, 80: 1039-1042.
[33] Häse, C.C., Finkelstein, R.A., (1993): Bacterial extracellular zinc-containing metalloproteases. Microbiological Reviews, 57: 823-837.
[34] Hernández-Apaolaza, L., Escribano, L., Zamarreño, Á.M., García-Mina, J.M., Cano, C., Carrasco-Gil, S., (2020): Root silicon addition induces Fe deficiency in cucumber plants, but facilitates their recovery after Fe resupply. A comparison with si foliar sprays. Frontiers in Plant Science, 11: 580552.
[35] Holmquist, B., Vallee, B.L., (1974): Metal substitutions and inhibition of thermolysin. Journal of Biological Chemistry, 249: 4601-4607.
[36] Hooper, N.M., (2002): Proteases: a primer. Essays in Biochemistry, 38: 1-8.
[37] Jacques, A., Latour, J.M., Sénèque, O., (2014): Peptide-based FeS_4 complexes: the zinc ribbon fold is unsurpassed to stabilize both the Fe^{II} and Fe^{III} states. Dalton Transactions, 43: 3922-3930.
[38] Janecek, S., (1994): Parallel beta/alpha-barrels of alpha-amylase, cyclodextrin glycosyltransferase and oligo-1,6 glucosidase versus the barrel of beta-amylase: evolutionary distance is a reflection of unrelated sequences. FEBS Letters, 353: 119-123.
[39] Jones, L.P., Hetreck, K.A., (1967): Silica in soils, plants, and animals. Advances in Agronomy, 19: 107-149.
[40] Katz, O., (2019): Silicon content is a plant functional trait: Implications in a changing world. Flora, 254: 88-94.
[41] Kaushik, P., Saini, D.K., (2019): Silicon as a vegetable crops modulator. Plants, 8: 148.
[42] Keilin, D., Hartree, E.F., (1945): Properties of catalase. Catalysis of coupled oxidation of alcohols. Biochemical Journal, 39: 293-301.
[43] Khajeh, K., Ranjbar, B., Naderi-Manesh, H., Habibi, A.E., Nemat-Gorgani, M., (2001): Chemical modification of bacterial α-amylases: changes in tertiary

structures and the effect of additional calcium. Biochimica et Biophysica Acta (BBA), 1548: 229-237.
[44] Kim, Y.H., Khan, A.L., Waqas, M., Lee, I.J., (2017): Silicon regulates antioxidant activities of crop plants under abiotic-induced oxidative stress. Frontiers in Plant Science, 8: 510.
[45] Kind, P.R.N., King E.J., Pash, V., Roman, W., Schmidt, W.E., (1954): Inhibition of enzymes by silicic acid. Biochemical Journal, 56: 45.
[46] Li, J., Leisner, S.M., Frantz, J., (2008): Alleviation of copper toxicity in *Arabidopsis thaliana* by silicon addition to hydroponic solutions. Journal of the American Society for Horticultural Science, 133: 670-677.
[47] Liang, Y., Nikolic, M., Bélanger, R., Gong, H., Song, A., (2015): Silicon-mediated tolerance to salt stress. In: Silicon in Agriculture, Dordrecht, pp. 123-142.
[48] Liang, Y., Sun, W., Zhu, Y.G., Christie, P., (2007): Mechanisms of silicon-mediated alleviation of abiotic stresses in higher plants. Environmental Pollution, 147: 422-428.
[49] Loew, O., (1901): Catalase, new enzyme of general occurrence, with special reference to the tobacco plant. United States Department of Agriculture Report, 68: 47.
[50] Lüthi-Peng, Q., Märki, H.P., Hadváry, P., (1992): Identification of the active-site serine in human pancreatic lipase by chemical modification with tetrahydrolipstatin. FEBS Letters, 299: 111-115.
[51] Ma, J.F., (2004): Role of silicon in enhancing the resistance of plants to biotic and abiotic stresses. Soil Science and Plant Nutrition, 50: 11-18.
[52] Ma, J.F., Takahashi, E., (1993): Interaction between calcium and silicon in water-cultured rice plants. Plant and Soil, 148: 107-113.
[53] MacAdam, J.W., Nelson, C.J., Sharpe, R.E., (1992): Peroxidase activity in the leaf elongation zone of tall fescue. Plant Physiology, 99: 872-878.
[54] Marklund, S., Marklund, G., (1974): Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. European Journal of Biochemistry, 47: 469-474.
[55] Martinez, M., Gómez-Cabellos, S., Giménez, M.J., Barro, F., Diaz, I., Diaz-Mendoza, M., (2019): Plant proteases: from key enzymes in germination to allies for fighting human gluten-related disorders. Frontiers in Plant Science, 10: 721.
[56] McDonald, C.E., Chen, L.L., (1965): The Lowry modification of the Folin reagent for determination of proteinase activity. Analytical Biochemistry, 10: 175-176.
[57] McGinnity, P., (2015): Silicon and its role in crop production. A Literature Review, 27p. (available: http://planttuff.com/wp-content/uploads/2015/12/silicon-agriculture-iiterature-rvw-1.pdf in nov. 2024)
[58] Mika, A., Buck, F., Lüthje, S., (2008): Membrane-bound class III peroxidases: identification, biochemical properties and sequence analysis of isoenzymes purified from maize (*Zea mays* L.) roots. Journal of Proteomics, 71: 412-424.
[59] Murali, A., Patel, S., (2017): The effect of different heavy metal acetate solutions on the inhibition of catalase enzyme. Journal of the South Carolina Academy of Science, 15: 68-74.
[60] Nakano, Y., Asada, K., (1981): Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplasts. Plant and Cell Physiology, 22: 867-880.
[61] Neumann, D., zur Nieden, U., (2001): Silicon and heavy metal tolerance of higher plants. Phytochemistry, 56: 685-692.
[62] Nikolic, D.B., Nesic, S., Bosnic, D., Kostic, L., Nikolic, M., Samardzic, J.T., (2019): Silicon alleviates iron deficiency in barley by enhancing expression of strategy II genes and metal redistribution. Frontiers in Plant Science, 10: 416.
[63] Ogawa, KI., Kanematsu, S., Asada, K., (1996): Intra-and extra-cellular localization of "cytosolic" CuZn-superoxide dismutase in spinach leaf and hypocotyl. Plant and Cell Physiology, 37: 790-799.
[64] Pavlovic, J., Kostic, L., Bosnic, P., Kirkby, E.A., Nikolic, M., (2021): Interactions of silicon with essential and beneficial elements in plants. Frontiers in Plant Science, 12: 697592.
[65] Pereira, A.S., Bortolin, G.S., Dorneles, A.O.S., Meneghello, G.E., Do Amarante, L., Mauch, C.R., (2021): Silicon seed priming attenuates cadmium toxicity in lettuce seedlings. Environmental Science and Pollution Research, 28: 21101-21109.
[66] Raven, E.L., Lad, L., Sharp, K.H., Mewies, M., Moody, P.C., (2004): Defining substrate specificity and catalytic mechanism in ascorbate peroxidase. Biochemical Society Symposia, 71: 27-38.
[67] Ray, R.R., Chakraverty, R., (1998): Extracellular β-amylase from *Syncephalastrum racemosum*. Mycological Research, 102: 1563-1567.
[68] Sarda, L., Desnuelle, P., (1958): Action de la lipase pancréatique sur les esters en émulsion. Biochimica et Biophysica Acta, 30: 513-521.
[69] Sarowar-Jahan, M.G., Shaela Pervin, M., Shariar Shovon, M., Dev Sharma, S.C., Roy, N., Rahman, M.H., (2012): Effect of metal ions, chelating agent and SH-reagents on radish (*Raphanus sativus* L.) Root β-Amylase. Journal of Stress Physiology and Biochemistry, 8: 180-188.
[70] Savvas, D., Ntatsi, G., (2015): Biostimulant activity of silicon inhorticulture. Scientia Horticulturae, 196: 66-81.
[71] Shiro, Y., Kurono, M., Morishima, I., (1986): Presence of endogenous calcium ion and its functional and structural regulation in horseradish peroxidase. Journal of Biological Chemistry, 261: 9382-9390.
[72] Shuster, L., Gifford, R.H., (1962): Changes in 3′-nucleotidase during the germination of wheat embryos. Archives of Biochemistry and Biophysics, 96: 534-540.
[73] Teschner, W., Schwarz, H.P., Madlener, R., Svatos, S., Pljevljakovic, A., Weber, A., (2011): Removal of serine proteases by treatment with finely divided silicon dioxide. Patent WO2011150284A2 (available: https://patents.google.com/patent/WO2011150284A2/en in nov. 2024)
[74] Tester, M., Davenport, R.J., (2003): Na^+ transport and Na^+ tolerance in higher plants. Annals of Botany, 91: 503-527.
[75] Tognolli, M., Penel, C., Greppin, H., Simon, P., (2002): Analysis and expression of the class III peroxidase large gene family in *Arabidopsis thaliana*. Gene, 288: 129-138.
[76] Torrance, J.W., MacArthur, M.W., Thornton, J.M., (2008): Evolution of binding sites for zinc and calcium ions playing structural roles. Proteins, 71: 813-830.
[77] Umemura, Y., Nishida, J., Akazawa, T., Uritani, I., (1961): Effect of silicon compounds on plant enzymes involved in phosphorus metabolism. Archives of Biochemistry and Biophysics, 92: 392-398.

[78] Verma, K.K, Song, X.P., Liang Q., Huang H.R., Bhatt, R., Xu, L., Chen, G.L., Li, Y.R., (2024): Unlocking the role of silicon against biotic stress in plants. Frontiers in Plant Science, 15:1430804.

[79] Welinder, K.G., (1992): Superfamily of plant, fungal and bacterial peroxidases. Current Opinion in Structural Biology, 2: 388-393.

[80] Wu, H., Si, Q., Liu, J., Yang, L., Zhang, S., Xu, J., (2022): Regulation of arabidopsis matrix metalloproteinases by mitogen-activated protein kinases and their function in leaf senescence. Frontiers in Plant Science, 8: 864-986.

[81] Yonetani, T., Ohnishi, T., (1966): Cytochrome C peroxidase, a mitochondrial enzyme of yeast. Journal of Biological Chemistry, 241: 2983-2984.

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