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Effects of Barium Stress in *Brassica juncea* and *Cakile maritima*: The Indicator Role of Some Antioxidant Enzymes and Secondary Metabolites

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Abstract: Soil contamination by toxic trace metal elements, like barium (Ba), may stimulate various undesirable changes in the metabolic activity of plants. The plant responses are fast and with, direct or indirect, generation of reactive oxygen species (ROS). To cope with the stress imposed by the ROS production, plants developed a dual cellular system composed of enzymatic and non-enzymatic players that convert ROS, and their by-products, into stable nontoxic molecules. To assess the Ba stress response of two *Brassicaceae* species (*Brassica juncea*, a glycophyte, and *Cakile maritime*, a halophyte), plants were exposure to different Ba concentrations (0, 100, 200, 300 and 500 µM). The plants response was evaluated through their morphology and development, the determination of plant leaves antioxidant enzymatic activities and by the production of plants secondary metabolites. Results indicated that the two *Brassicaceae* species have the ability to survive in an environment containing Ba (even at 500 µM). The biomass production of *C. maritima* was slightly affected whereas an increase in biomass *B. juncea* was noticed. The stress imposed by Ba activated the antioxidant defense system in the two species, noticed by the changes in the leaves activity of catalase (CAT), ascorbate peroxidase (APX) and guaicol peroxidase (GPX), and of the secondary metabolites, through the production of total phenols and flavonoids. The enzymatic response was not similar within the two plant species: CAT and APX seem to have a more important role against the oxidative stress in *C. maritima* while in *B. juncea* is GPX. Overall, total phenols and flavonoids production was more significant in the plants aerial part than in the roots, of the both species. Although the two *Brassicaceae* species response was different, in both plants catalytic and non-catalytic transformation of ROS occurs, and both were able to overcome the Ba toxicity and prevent the cell damage.

Keywords: *Brassica juncea*; *Cakile maritima*; antioxidant enzymes; barium stress; oxidative stress; secondary metabolites

1 Introduction

The increase in anthropogenic activities such as intensive agriculture, metallurgical industries, heavy traffic, atmospheric deposition, mining and dumping of waste generate an increase in the concentration of several toxic trace metal elements (TME) such as mercury, lead, cadmium, etc. beyond optimal levels in soil, water and air [1]. Pollution by TME presents a significant problem for ecological systems because they are not degraded by chemical or biological processes, which makes them difficult to eliminate from the environment [2]. The tolerance or sensitivity to abiotic stress depends on the type of stress, the duration of the stress, type of plant species as well as the stage of development of the plant [3].

Soil contamination by TME can induce many undesirable changes in the metabolic activity of plants. Barium (Ba) is one of these contaminants. Over the past 40 years, Ba has been used extensively in the industrial and medical fields, particularly in the petroleum industry, the steel industry and as an agent for radiography of the gastrointestinal tract (GI) [4]. Barium is considered the 14th most abundant element on earth's crust. This element soil contents ranges between 265 and 835 $\mu g.g^{-1}$ dry weight (DW), depending on the type of soil [5]. In fact, Ba has been reported to have several toxic effects on the environment [6] and can exist in the form of $BaSO_4$, $BaCO_3$, $BaHCO_3$, $BaNO_3$, $BaCl_2$, $BaCl^+$, $BaOH^+$, BaF^-, $BaB(OH)^{4+}$, Ba^{2+} and $Ba(CH_3COO)_2$ [7]. Moreover, Ba is not considered essential for organisms and can be toxic for animals and plants in high concentrations [8]. The toxicity of Ba is linked to its solubility, the more soluble it is the more toxic it becomes.

It has been reported that as free ionic metal, Ba^{2+}, Cd^{2+} and other metallic elements can modify plant performance from the subcellular level to the ecosystem level [9]. Similarly, the interaction of free ionic metals with cellular components can trigger a variety of metabolic responses in seconds, sometimes with direct or indirect generation of reactive oxygen species (ROS) [10].

One of the promptest consequences when plant cells are exposed to toxic concentrations of TME is the production of ROS, i.e., hydroxyl radicals ($^\bullet OH$) and superoxide ($O^{\bullet -2}$), as well as non-radicals, such as hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$) [11] and are highly reactive and deleterious and could cause damage to nucleic acids, proteins and lipids [12]. However, plants develop several adaptation mechanisms in response to environmental stress. Plants have developed a dual cellular system composed of enzymatic and non-enzymatic players to recover/balance the ROS production [12]. The enzymatic mechanism comprises enzymes like superoxide dismutase (SOD), ascorbate and guaiacol peroxidase (APX, GPX), glutathione-S-transferases (GST) and catalase (CAT). The non-enzymatic mechanism is the non-enzymatic response regulating the production of polyamines, as well as low molecular weight compounds, such as reduced glutathione (GSH), vitamins (such as ascorbic acid and α-tocopherol), osmolytes (like proline) and secondary metabolites, notably flavonoids, phenolics and carotenoids [13]. Indeed, the activation of cells antioxidant system is a key element in the plants tolerance to metals [14]. The accumulation of secondary metabolites in plants generally occurs under different types of stress [15] such us TME which will be chelated by secondary metabolites [16].

Lajayer et al. [17] reported that following exposure to TME stress, the production of secondary metabolites increased. Among these secondary metabolites are phenols and flavonoids. Enhanced biosynthesis of phenolics helps protecting plants from oxidative stress after exposure to metallic stress [18–20]. Phenolics can chelate heavy metals [21]. Several studies have reported the increase in total phenols in *Brassica juncea* following exposure to Cu [22], Cr [18], Cd [23], Pb [24] and in *Fagopyrum esculentum* exposed to Al [25] as well as in *Zea mays* following exposure to Cu, Pb and Cd [26]. Flavonoids have been reported to enhance the process of metal chelation, which helps in reducing the levels of harmful hydroxyl radicals in plant cells [27], and it has been observed that flavonoid levels in plants have found to be enhanced by an excess of metal [20,28]. However, the flavonoid content was increased in *Brassica juncea* exposed to Pb [19,24] and in *Withania somnifera* exposed to Cd [29].

In this sense, the aim of this study was to evaluate the stress response of two *Brassicaceae* (*Brassica juncea* and *Cakile maritima)* after Ba exposure. The effect of the different Ba concentrations on plants morphology and development, enzymatic activity and the production of secondary metabolites have been studied.

2 Materials and Methods

2.1 Plant Culture

Seeds of *Brassica juncea* and *Cakile maritima* were disinfected with calcium hypochlorite for 15 minutes. The disinfected seeds were rinsed thoroughly and soaked in distilled water for 2 h. The seeds were then placed in plastic pots containing a mixture of perlite/gravel (2:1, v/v) as a substrate and were daily irrigated with tap water. After germination, the seedlings were irrigated with a Hewitt [30] nutritive solution (pH was between 6.5 and 7) until the beginning of treatment. After 4 weeks, the uniform plants were selected for the experiments and divided into 5 groups of twelve plants for each species. The plants were exposed regularly (3 times a week) to different concentrations of Ba supplied as $BaCl_2$ (0, 100, 200, 300 and 500 µM) added into the nutrient solution. Experiments were performed in a greenhouse under semi-controlled conditions with a natural photoperiod (14 h light), mean temperature $25 \pm 5°C$, and relative humidity between 60 and 90% (Fig. 1).
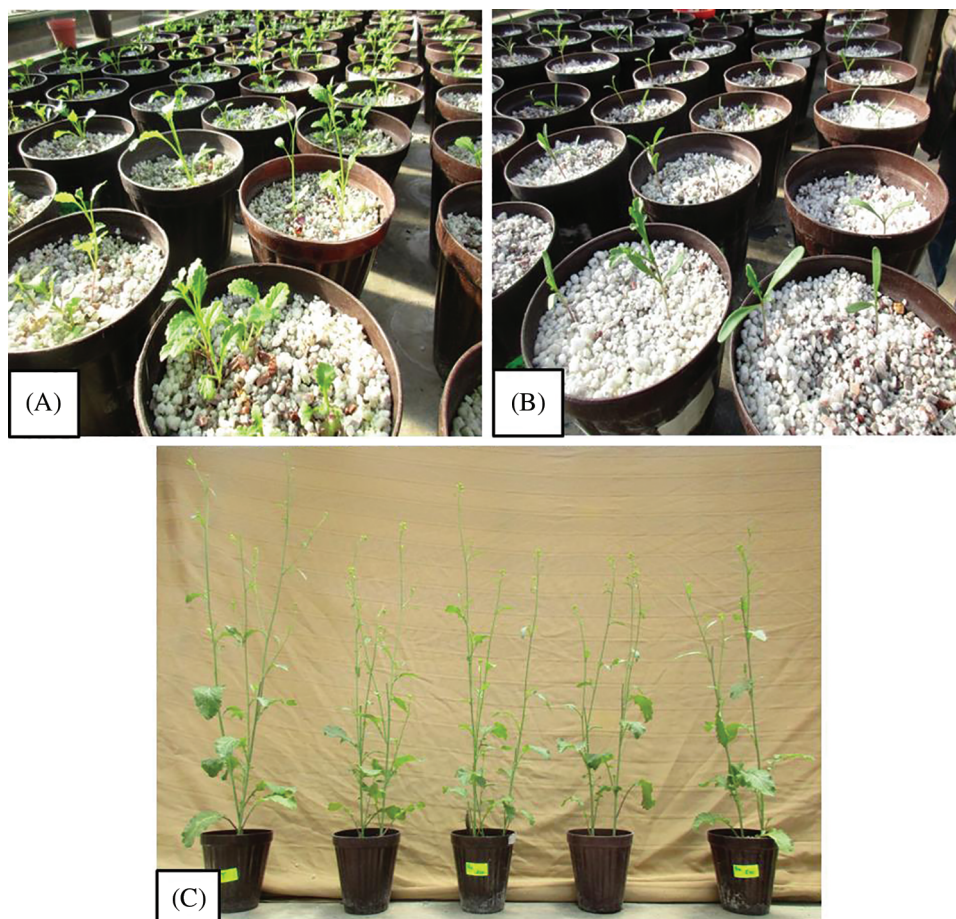

Figure 1: Photo of the culture at beginning of treatment (*Brassica juncea* (A), *Cakile maritima* (B)) and at end of treatment (*Brassica juncea* (C))

2.2 Plant Harvesting

After 45 days, the plants were separated into 2 lots of 6 plants. The first lot was used for the measurement of the enzyme activities. Shoots (stem and leaves) were frozen in liquid nitrogen and stored at −80°C. For the second batch, shoots were separated from the roots. To remove traces of metal adsorbed on the root's surfaces, these organs were dipped in a cold solution of $CaCl_2$ for 5 min [31] and afterwards rinsed 3 times with cold distilled water. The fresh weight (FW) was immediately determined and dry weight (DW) was measured after oven drying at 60°C until constant weight.

The tolerance index (TI), which is a measure of the tolerance of the plant to TME, was determined by comparing the dry biomass of plants subjected to metal treatment with the control as described by Sleimi [32]:

Tolerance index = (Biomass of treated plants/Biomass of control plant) × 100.

2.3 Enzyme Activities

Leaves (0.4 g) were ground in a mortar, in 2 mL of potassium phosphate buffer (pH 7.0), containing Na-ascorbate (5 mM) and EDTA (0.2 mM). The mixture was filtered through four layers of mir-a clothon and centrifuged at 6000 rpm for 15 min at 4°C. The resulting supernatant was used as a crude enzyme extract. The activities of CAT (EC1.11.1.6), GPX (EC1.11.1.7) and APX (EC1.11.1.11) were determined according to Aebi [33], Fielding et al. [34] and Nakano et al. [35], respectively. The details of methods were described in detail by Sleimi et al. [36].

2.4 Total Phenols and Flavonoids Level Assay

Approximately 30 mg of dry matter (shoots/roots) was added to 10 mL a methanol/water mixture (80/20; v/v). The macerate was kept overnight, centrifuged at 2500 rpm for 30 min and then filtered. For the determination of polyphenols, the method of Velioglu et al. [37] was followed. A 100 µl of the macerate was mixed with 500 µL of Folin-Ciocalteu. After 5 min of incubation in the dark, 400 µl of Na_2CO_3 at 7.5% was added. After 90 min incubation in the dark and at room temperature, the mixture was centrifuged at 5000 rpm for 3–4 min and the supernatant absorbance was measured at 765 nm. Gallic acid was used as standard reference for the quantitative estimation. The dosage of flavonoids was determined from the spectrometric method adapted by Quettier-Deleu et al. [38]. To 500 µl of macerate, 500 µl of freshly prepared methanoic solution of 2% $AlCl_3$ was added and incubate for 30 min. The measurement was done at 430 nm. Rutin was used as standard reference for the quantitative estimation.

2.5 Statistical Analysis

All data presented are the mean values of 6 replicates, which are presented along with the standard error (±) in bars in the figures and table. The effect of TME on the variability of the response parameters was assessed using regression analyses and One-way ANOVA. Statistical analyses were done using Statistica 8. Tukey's HSD test was performed to define which specific mean pairs were significantly different ($p < 0.05$).

3 Results

3.1 Plant Morphology

The Ba effects on the development of *Brassica juncea* and *Cakile maritima* were evaluated based on the production of fresh biomass and are presented in Tab. 1. Results showed an opposite effect of Ba on the production of fresh biomass in these two *Brassicaceae*. In fact, the used Ba concentrations promoted the growth of *B. juncea*, whereas for *C. maritima* a decrease in the production of fresh biomass was observed. For *B. juncea* an increase in the production of fresh biomass was noticed in the whole plant. It varied from 8677 mg in the control to 13488 mg for 100 µM of Ba and 9568 mg for the high concentration (500 µM) (Tab. 1).

Table 1: Fresh weight (shoots, roots and whole plant), shoots-roots ratio (S/R) and tolerance index (TI) of *B. juncea* and *C. maritma* in response to different concentrations of Ba. Values with different letters are significantly different at $p \leq 0.05$

Treatment Ba (µM)	Shoots FW (mg)	Roots FW (mg)	Whole plants FW (mg)	S/R	Shoots TI (%)	Roots TI (%)	Whole plants TI (%)
Brassica juncea							
Control	7077 ± 248[a]	1600 ± 117[a]	8677 ± 309[a]	4.5 ± 0.3[a]			
100	9471 ± 1265[a]	4017 ± 338[b]	13488 ± 1477[b]	2.4 ± 0.3[b]	138 ± 20[a]	272 ± 24[a]	163 ± 19[a]
200	9368 ± 1092[a]	2704 ± 404[a]	12073 ± 1472[ab]	3.6 ± 0.3[ab]	137 ± 17[a]	178 ± 29[b]	144 ± 19[a]
300	8087 ± 340[a]	2119 ± 67[a]	10206 ± 344[ab]	3.8 ± 0.2[a]	116 ± 6[a]	137 ± 5[b]	120 ± 4[a]
500	7671 ± 465[a]	1897 ± 249[a]	9568 ± 645[ab]	4.3 ± 0.4[a]	109 ± 7[a]	121 ± 18[b]	112 ± 8[a]
Cakile maritima							
Control	15059 ± 2110[a]	1074 ± 168[a]	16134 ± 2223[a]	15.0 ± 1[a]			
100	12166 ± 773[a]	1018 ± 98[a]	13183 ± 788[a]	12.0 ± 1[a]	80 ± 5[a]	94 ± 11[a]	81 ± 5[a]
200	11078 ± 2026[a]	1217 ± 216[a]	12294 ± 218[a]	9.4 ± 1[a]	72 ± 14[a]	116 ± 24[a]	75 ± 14[a]
300	10543 ± 1271[a]	1179 ± 238[a]	11722 ± 1415[a]	10.0 ± 1[a]	69 ± 9[a]	112 ± 26[a]	71 ± 9[a]
500	13019 ± 1574[a]	1339 ± 132[a]	14358 ± 1688[a]	9.7 ± 1[a]	86 ± 11[a]	129 ± 14[a]	88 ± 11[a]

For the roots, the plants treated with 100 µM Ba showed the highest biomass compared to the control. An increase of 2.51 fold was noticed. Even with the highest concentrations, there was an increase in the production of root fresh biomass.

The variation of the fresh biomass of *C. maritima* exposed to the different concentrations of Ba showed a decrease in the shoots biomass. For the whole plant, the production decreased with increasing the concentration (100, 200, 300 µM) but it increased again with 500 µM of Ba. However, roots showed an increase in the production of fresh biomass. The highest value was 1339 mg with 500 µM and 1074 mg in the control. Nevertheless, the deviations observed compared to the control were not significant (Tab. 1).

The TI values decreased in *C. maritima* in the aerial parts, showing a decrease in the biomass. Whereas, *B. juncea* noted an increase in TI in the aerial parts and the roots.

3.2 Enzyme Activities

The CAT, GPX and APX activities (expressed in Δ OD.min^{-1}.g^{-1}) are presented in the Figs. 2, 3 and 4 respectively.

The exposure of 200, 300 and 500 µM of Ba decreased the CAT activity in the leaves of *B. juncea*, reaching almost half in the 500 µM comparing to the control. No significant differences were found between the control plants (79 Δ OD.min^{-1}.g^{-1}) and those treated with 100 µM Ba (80 Δ OD.min^{-1}.g^{-1}) (Fig. 2A).

Unlike *B. juncea*, CAT activity in *C. maritime* leaves increased significantly from 51, in the control, to 158, 129 and 145 Δ OD.min^{-1}.g^{-1} in the groups 200, 300 and 500 µM, respectively, while the group exposed to 100 µM of Ba (35 Δ OD.min^{-1}.g^{-1}) showed no significant differences relative to the control (Fig. 2B).

Generally, treatment of *B. juncea* with Ba did not affect APX activity. A slight increase in APX activity was observed in the 300 and 500 µM groups, but not statistically significant ($p < 0.05$) as shown in Fig. 3A. All the Ba concentrations used stimulated the APX activity compared to the control in *C. maritime* leaves (Fig. 3B). APX activity showed no statistically significant differences among the groups treated with Ba ($p < 0.05$), with mean values of 0. 09 Δ OD.min^{-1}.g^{-1} for the control group and 0.18, 0.17, 0.17 and 0.18 Δ OD.min^{-1}.g^{-1} for the 100, 200, 300 and 500 µM groups, respectively.
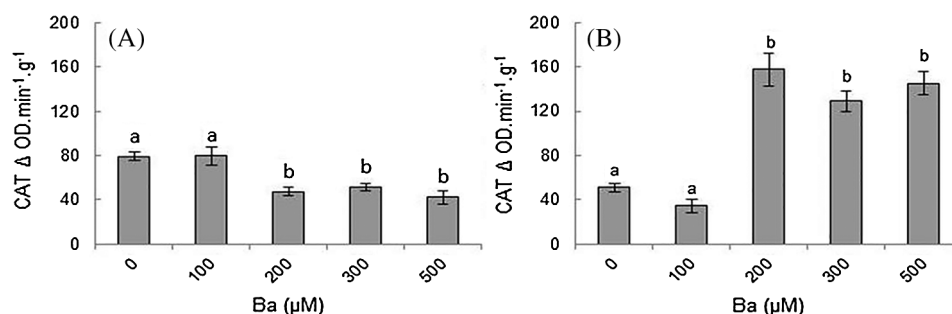
Figure 2: Catalase (CAT) activity in the leaves of *B. juncea* (A) and *C. maritima* (B) exposed to barium (Ba). Bars marked with different letters are significantly different at $p < 0.05$

Figure 3: Ascorbate Peroxidase (APX) activity in the leaves of *B. juncea* (A) and *C. maritima* (B) exposed to barium (Ba). Bars marked with different letters are significantly different at $p < 0.05$
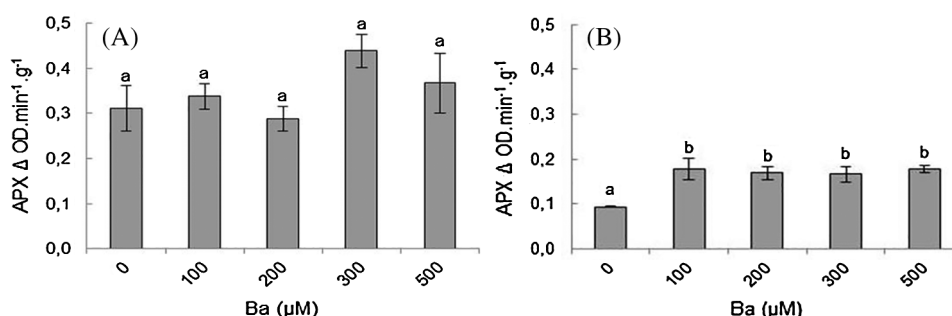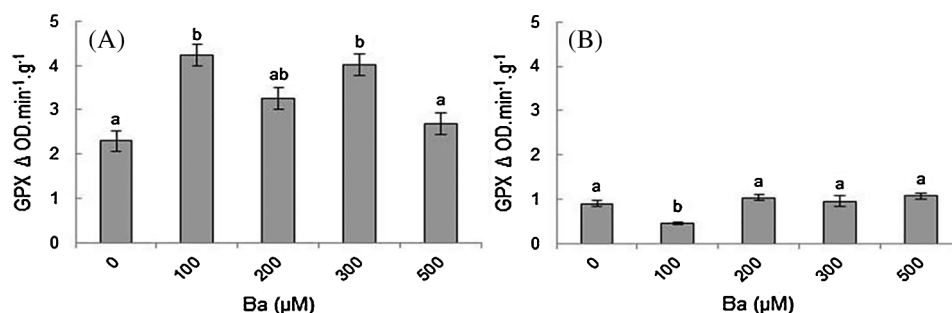

Figure 4: Guaiacol Peroxidase (GPX) activity in the leaves of *B. juncea* (A) and *C. maritima* (B) exposed to barium. Bars marked with different letters are significantly different at $p < 0.05$

GPX activity showed an increase with all Ba concentrations in *B. juncea* leaves except in the 500 μM group. The highest activity was noted in the leaves of plants exposed to 100 and 300 μM, while the lowest GPX activity was observed in the 500 μM group (2.7 Δ OD.min^{-1}.g^{-1}) (Fig. 4A). In *C. maritima*, GPX activity did not show any variation following the treatment with Ba, with the exception of treatment with 100 μM. The addition of 100 μM of Ba decreased the GPX activity from 0.9 in the control to 0.46 Δ OD.min^{-1}.g^{-1}.

3.3 Total Phenols and Flavonoids Level

The addition of Ba to the irrigation solution induced a significant increase in the production of total phenols (TP) in the aerial parts of both plants treated (Fig. 5). The production of TP in response to stress

by Ba showed a clear difference between shoots and roots. It increased from 7.5 in the control to 18 mg.g^{-1} (DW) with the highest concentration of Ba in *B. juncea* shoot. On the other hand, when compared with the control, no significant variation ($p \leq 0.05$) was observed in the production of TP in *B. juncea* roots.
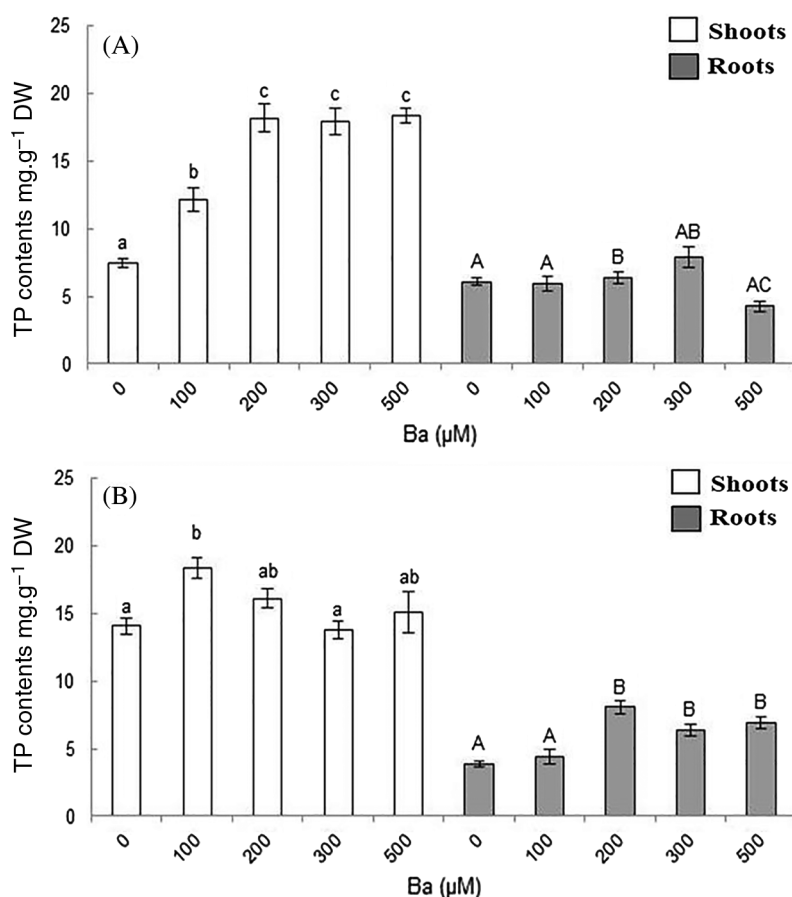

Figure 5: Total phenols (TP) content in shoots and roots in *B. juncea* (A) and *C. maritima* (B) subjected to barium (Ba) stress. Values with different letters are significantly different at $p \leq 0.05$

Results showed that there was, in general, an increase in the TP content in the aerial parts of *C. maritima*. Except with the 300 μM concentration, which showed no variation comparing to the control. The maximum production (18 mg.g^{-1} DW) was noted in the plants treated with 100 μM of Ba.

For roots of *C. maritima*, with the exception of the 100 μM of Ba, a stimulation of TP production was observed with the increase of Ba concentration in the irrigation solution (Fig. 5B). Values observed were 3.84, 8.1, 6.36 and 6.94 mg.g^{-1} DW in the control, 200 μM, 300 μM and 500 μM, respectively.

The variation of flavonoids content was considered non-significant in *B. juncea* (Fig. 6A). In the aerial parts, the highest content was observed with 200 μM of Ba (12 mg.g^{-1} DW) comparing to the control (9.4 mg.g^{-1} DW). Flavonoids production did not show any significant variation in the roots of *B. juncea* compared to the control (Fig. 6A).

The treatment of *C. maritima* with Ba showed that the concentration 100 μM stimulated the production of flavonoids in the plant. The flavonoids content increased from 9.1 to 12 mg.g^{-1} DW in the aerial parts, and it decreased with the other concentrations compared to the control. In the roots, the values vary from 1.4 in the control to 1.6 mg.g^{-1} DW with 100 μM. The values were lower than the control for the other doses.
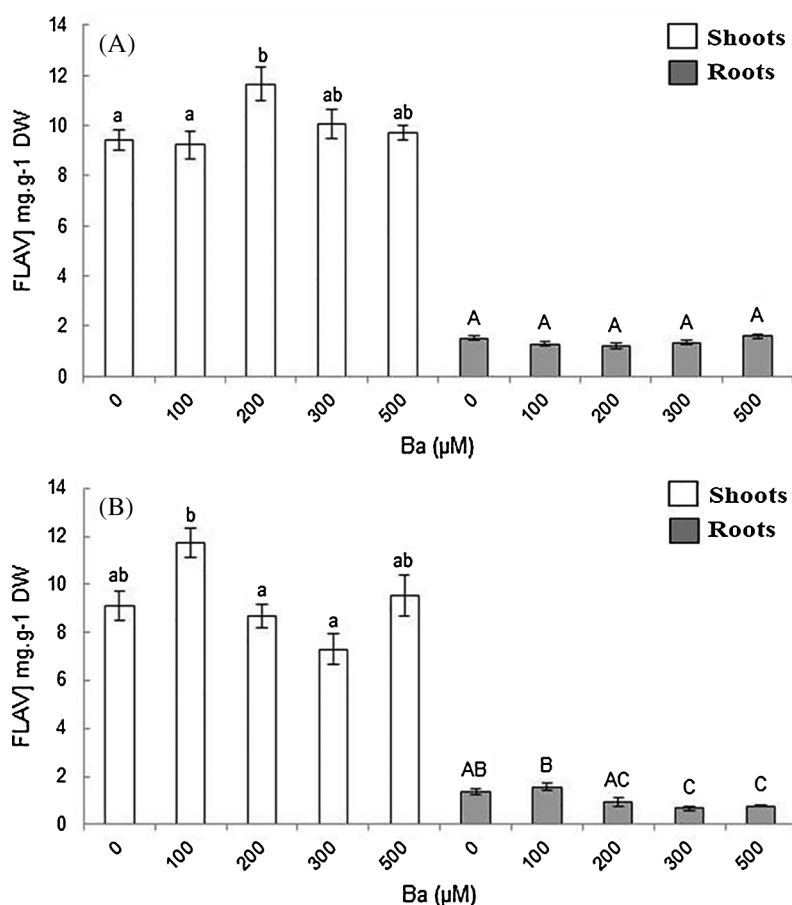
Figure 6: Variation of flavonoid content in the shoots and roots in *B. juncea* (A) and *C. maritima* (B) treated with different concentrations of barium (Ba). Values with different letters are significantly different at $p \leq 0.05$

4 Discussion

Information on the toxicity of Ba to plants is limited. However, many studies [39,40] suggest that Ba is toxic to plants. In the present study, we tested the effect of 100, 200, 300 and 500 µM of Ba on plant growth, some antioxidant enzymes activity response and the production of secondary metabolites on *B. juncea* and *C. maritima*. The results indicate that *B. juncea* tolerates Ba better than *C. maritime*, based on plant biomass.

Scientists consider the biomass of shoots as a determinant of the plant's capacity to tolerate the stress of TME [41]. The fresh biomass of aerial parts of two *brassicaceae* studied were not affected in the same way under Ba stress. Comparing with the control, the shoots biomass of *B. juncea* increased even with the highest concentration of Ba (500 µM), while *C. maritima* has shown a decrease in its fresh biomass. But it should be mentioned that statistically, this decrease was not significant. The effect of Ba on the development of plants may vary depending on the species. It has been reported that Ba caused a reduction in the production of bean biomass [39] and soybeans [40]. On the other hand, Coscione et al. [42], with concentrations up to 300 mg. Kg^{-1} of Ba, found no toxic effect on plants cultivated in a rhodic hapludox.

Our results showed that all the concentrations of Ba used in the assays had a positive effect on the development of *B. juncea* mainly at 100 and 200 µM. Suwa et al. [40] noted that 100 µM Ba significantly inhibited growth and photosynthetic activity of soybean leaves. They explained the negative effect of Ba on reduced plant growth by reduced assimilation of CO_2 caused by limited photosynthetic activity.

Both *B. juncea* and *C. maritima* were able to maintain good growth and survive under Ba stress. Our results are supported by previous studies which reported that *B.* juncea [43] and *C. maritima* [44] are capable of growing in soils contaminated with heavy metals. These authors reported that *B. juncea* tolerated and even grew well at high doses of zinc, while *C. maritima* is a cadmium tolerant plant. The tolerance of plants to TME may be linked to the development of mechanisms such as the sequestration of metals in tissues or cells compartments [45], which implies a restriction of the two upward movements in the shoots (avoidance mechanism) and translocation of excess metals into the leaves [46].

The purpose of TI is to characterize plant tolerance. Ghosh et al. [47] defined the plant's TI by its capacity to grow well and to tolerate a large amount of concentration of metals. The TI values of *B. juncea* showed an increase in the biomass of the aerial parts and of the roots, thus proving tolerance to Ba, whereas in *C. maritima*, the TI showed a decrease, except with 200, 300 and 500 µM at the level of the roots. Despite this decrease, *C. maritima* survived until the end of treatment. These results suggest that *B. juncea* tolerates Ba better than *C. maritima*.

In response to abiotic stress, plants typically produce ROS [48]. ROS are rapidly detoxified by cellular enzymes which can mitigate the caused oxidative damage. In our study, CAT was clearly activated in the leaves of *C. maritima* since its activity showed a clear increase with the high Ba doses used. Our results are in agreement with Melo et al. [49] who observed an increase in this enzyme in *Glycine max*. L. stressed by Ba. It was clear that this increase in the activity of CAT has been triggered in response to a production of ROS induced by Ba stress. But we did not obtain the same response of CAT in *B. juncea*. Ba treatment decreased CAT activity except with the low dose (100 µM). This was probably due to the effect of high Ba concentrations. Rastgoo et al. [50] reported that CAT was not able to protect cells against ROS under high concentration of TME. The decrease in some enzyme activities at high concentrations of TME may be due to an imbalance in metabolism and the generation of ROS [51].

The APX activity showed the same reaction to metallic stress in the two *Brassicaceae*. Nonetheless, the APX activity increased slightly but not significantly in *B. juncea*, in response to all the concentrations of Ba used, except with 200 µM in *B. juncea*. Whereas in *C. maritima*, a significant increase in APX activity was noticed for all Ba concentrations. In fact, APX and GPX, powerful H_2O_2 scavengers, seem to maintain its levels because the uncontrolled export of this toxic ROS from organelles to the cytosol can have an undesirable effect due to the formation of hydroxyl radicals by the Haber-Weiss reaction catalyzed by a metal [52].

The accumulation of TME in plants generates an inhibition or stimulation of the activity of various antioxidant enzymes [53]. A clear decrease in GPX activity was observed with 100 µM in *C. maritima*. However, GPX activity increased following Ba stress, mainly with 100 and 300 µM in *B. juncea*. Also, Al Mahmud et al. [54] reported that GPX and APX activity had increased in *B. juncea* subjected to stress by Cd. Therefore, results indicate that a high activity of CAT and APX can serve as a better defense tool to resist the oxidative stress induced by Ba in *C. maritime*, while in *B. juncea*, the APX and GPX activity play the defense role against oxidative stress.

The difference in the activity of antioxidant enzymes in plants under environmental stress such as TME, depends on the species (physiological and genetic potential of the plant), the duration of treatment as well as the concentration of TME [55]. Stress caused by TME can lead an increase in ROS production, which interacts with various molecules, leading to lipid peroxidation and damage to the cell membrane system [52].

Phenols have the ability to slow down the formation of free radicals [56]. The metal chelating and ROS scavenging properties of phenols and complex forming property of flavonoids protects the plants from the effects of TME [57]. Metal ions including nickel, lanthanum, europium, silver and cadmium, and oxalate have been influenced the secondary metabolite production in various plants [58]. Our results show a significant increase in the TP content in *B. juncea* leaves with 500 µM of Ba. In fact, a 2.45 fold increase of the TP content in the aerial parts occurred compared to the control. In the root, TP content had a slight

increase followed by a decrease with the highest concentration of Ba (500 μM). The increase in TP content under metal stress has been mentioned in other studies. Handa et al. [59] found that the content of total polyphenols was significantly increased in *B. juncea* under the influence of Cr (an increase of 44.9% in level of total phenols was observed in Cr treated plants compared to control).

Also our results are in agreement with earlier works conducted on *Lactuca sativa* and *Brassica napus* treated with waste water contaminated by TME where the contents of phenols were increased [60]. *Lycopersicon esculentum* subjected to Cu stress were also reported to have enhanced levels of phenols [61]. Under Ba stress, the aerial parts of *C. maritima* did not show a significant increase in TP content. This can be explained by the fact that this halophyte used other defense mechanisms against the stress caused by Ba. However, the TP content was significantly increased at roots compared to the control.

The role of secondary metabolites in counteracting ROS stress is well documented [62]. In plants, phenolic compounds have a special chemical structure in plants which protect cells against oxidative stress by chelating metals and binding to free radicals accompanied by less lipid peroxidation [57]. Ma et al. [63] reported that there is a direct correlation between TME and the expression of phenylalanine ammonia lyase (PAL), the first gene intervening in the phenylpropanoid pathway. PAL is a key enzyme in the biosynthesis of phenolic compounds such as flavonoids and anthocyanins, which enhances the increase in phenolics and flavonoids in response to certain biotic and abiotic stresses [64]. These data may explain the accumulation of phenolic acids in plant cells stressed by TME. Handa et al. [59] reported that enhanced expression of PAL could be the reason for increased accumulation of total phenols and flavonoids in *B. juncea* plants under Cr stress.

Flavonoids inhibit lipid peroxidation and improve the stability and fluidity of the membrane, preventing the release of ROS and the inhibition of peroxidation reactions [65]. In our study, only with 200 μM of Ba, a significant increase in the flavonoid content was observed, in relation to the control, in the aerial parts of *B. juncea*. Even in the root, treatment with Ba did not modify the production of flavonoids. Generally, the same behavior was observed in *C. maritima* stressed by the Ba. Only a significant variation in flavonoid content in the aerial parts was noted with 100 μM of Ba, compared to the control, while there is a significant decrease with 300 and 500 μM in the roots. These results are in agreement with the studies carried out by Babula et al. [66]. In their work they observed a decrease in flavonoids in the shoots and roots of *Hyperium perforatum* in response to the stress of lanthanum and cadmium. Berni et al. [67] explained the non-intervention of flavonoids in the defense against oxidative stress in these studies, by the fact that plants can rather invest first in the pathway to produce phenolic acids (hydroxycinnamic acids) and do not activate genes involved in the following steps (which lead to the formation of flavonoids and anthocyanins) to save energy, while being able to counter stress via phenolic compounds.

Generally, the application of stress by the Ba on the two plants has helped to activate the anti-oxidant defense system: Enzymatic system (increase in the activity of enzymes like CAT, APX and GPX) and non-enzymatic system (production of TP and flavonoids). The production of antioxidant enzymes and certain secondary metabolites may present a defense mechanism for these two *brassicaceae* against metal stress.

5 Conclusion

C. maritima and *B. juncea*, can cope and survive in an environment containing Ba. Although biomass production of *C. maritima* was slightly affected by Ba, *B. juncea* showed an increase in biomass. It seems that *B. juncea* tolerates Ba better than *C. maritima*. The ability of two brassicaceae to survive in an environment contaminated with this element may be linked to the development of some defense mechanisms against stress induced by TME, such as, the stimulation of the activity of some antioxidant enzymes as well as the production of secondary metabolites. However, the study of other tolerance parameters such as lipid peroxidation and the measurement of toxicity parameters (e.g., endogenous

H$_2$O$_2$ and Hydroxyl radicals) may help to better understand the mechanism of tolerance and/or adaptation of the two *brassicaceae* species to Ba stress.

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