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Iron Deficiency Modulates Secondary Metabolites Biosynthesis and Antioxidant Potential in *Sulla carnosa* L. Primed with Salicylic Acid

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Abstract: Iron (Fe) is a vital nutrient for the development of many plants. Therefore, enhancing plant performance and production in relation to Fe deficiency is becoming a serious challenge. In this work, we intended to survey the effect of seed pretreatment with salicylic acid (SA) on oxidative status, phenolic composition, and related antioxidant activities in two varieties of *Sulla carnosa* (Sidi Khlif: SK and Kalbia: KA) treated with different Fe concentrations. In unprimed plants, the levels of phenolic compounds were very distinguishable among the two varieties, being superior in KA compared to SK. Interestingly, priming KA seeds with SA under control conditions or deficient conditions (D+SA treatment) caused a decreasing tendency in the contents of total phenolic compounds (TPC) and total flavonoids (TFC), whereas an opposite behavior was observed in SK. Moreover, an improvement of hydroxycinnamic and flavonoid groups, in addition to antioxidant activities (TAC, DPPH$^{\bullet}$, β-carotene and FRAP), was markedly found in SK primed with SA. According to these findings, SA seed pretreatment had a beneficial effect on the metabolic performance of this species under different Fe supply, regardless of whether the intensity of improvement was related to Fe concentration applied, variety, as well as plant organ. The results suggest that SA can account for the effective modulation of the secondary metabolites metabolism in *S. carnosa* plants to deal with the detrimental impacts of Fe deficiency.

Keywords: Fe deficiency; biotechnological tool; salicylic acid; UHPLC-DAD-ESI/MS^{n}; phenol compounds; antioxidant activities

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1. Introduction

Iron (Fe) is one of the main globe crust micronutrients. It is required in many processes, e.g., electron or oxygen transfer, photosynthesis, respiration, DNA synthesis, and N_2 fixation [1]. Its deficiency is a prevalent disorder in plants, especially in those grown in alkaline soils. Thus, Fe deficient species are often unable to preserve Fe homeostasis, due to a restricted soluble portion of this element in the soil [2]. As an example, in Tunisia (North and Center), calcareous soils containing bicarbonate predominate [3]. Under these conditions, the Fe absorption by plant roots and its transport towards shoots are reduced due to the decrease of its reduction mechanism [4].

To solve this problem, Fe deficient plants adopted two different strategies to facilitate Fe acquisition from the rhizosphere, particularly in calcareous soils. With this respect,

two mechanisms of Fe acquisition are revealed by Fe resistant genotypes, i.e., strategy I and strategy II. Strategy II is exhorted only by the Poaceae group, and it is based on the release by roots of Fe (III)-solubilizing compounds known as phytosiderophores (PS) [5]. In turn, strategy I plants belong to the non-graminaceous monocotyledons and dicotyledons species which are characterized by the increase of Fe reduction by plant roots owing to the stimulation of rhizosphere acidification, in addition to the accumulation of organic acids and/or release of chelating compounds, particularly phenolics [6]. However, it is crucial to note that these chelating compounds, such as organic acids, flavins, and phenolic compounds, play a crucial role in the mobilization of Fe in strategy I plants [7]. These bioactive metabolites are considered as efficient Fe chelators by inducing the dissolution of ferric hydroxide unavailable for plants in the rhizosphere [1,3].

On the other hand, it is well known that Fe can display oxidative stress in plants, when present at both scarce and poisonous levels, essentially by damaging the functionality of the mitochondria photosynthetic chain [7], which boosts the formation reactive oxygen species [8]. To sustain damage from oxidative stress, plant resort to defense mechanisms, requiring namely enzymatic and non-enzymatic antioxidants [1,5]. Among these substances, polyphenols are mainly relevant due to their significant antioxidant potential, in addition to their chelating properties. According to recent investigations [1,6], these compounds produced in different plant tissues notably contributed to preserve reactive ROS under the toxic levels during abiotic/biotic stresses.

Phenols content and composition can be affected when plants are subjected to biotic and abiotic restrictions [9]. In fact, environmental restrictions commonly have an opposite impact on the levels of phenolic compounds. The accumulation is usually accompanied by plant biomass decrease, essentially at the shoot level. Moreover, it is expected that the optimal yield of polyphenols is a typical feature of stress-tolerant species [10]. As expected by Zocchi et al. [4], under Fe deficiency conditions as well as other metals, the accumulation of such metabolites varied according to the plant ability to stimulate numerous mechanisms, in addition to the stress intensity. Phenolic compounds are claimed to be good Fe chelators, in addition to other metals, and even improve plant growth and water status, by interacting with other hormones, such as auxins and gibberellins [11,12].

Furthermore, the scientific interest focused on halophytes has expanded in the last years under the challenge of climate alterations, due to their significant contribution in the improvement of crops adaptation in soils affected by salt. Halophytes are also traditionally consumed for their organoleptic properties. In particular, the innovative approach of the supposed "cash crop halophytes" added economic incomes, ensuring food and nutrition security, in addition to fiber and industrial uses [13]. Among these halophyte species, Sulla plants constitute an important genetic contribution to pastoral production, characterized by their efficiency to water stress and soil fortification owing to their power of nitrogen fixing [14]. It has also been established that bacteria inhabiting the rhizosphere of *Sulla carnosa* seems to be a promising source for stimulating plant growth under high salinity conditions, showing high salt tolerance and exhibiting a multiple set of plant growth promoting (PGP) activities [14]. In addition, the genus Sulla contains a variety of chemical constituents that are reputed for their biological properties [15].

At present, the majority of plant species developed for industrial and food purposes are reputed for their susceptibility to environmental constraints. To overcome this difficulty, farmers stopped using Fe(III)chelates, which are very expensive and harmful to the fertile soil [16]. According to many studies, the application of the priming seeds technique can be considered an alternative approach for providing optimal plant growth and crop yield while respecting the environment [17]. Among the efficient priming compounds, salicylic acid (SA) has been the most studied. Indeed, SA is an endogenous growth regulator belonging to the group of phenolic compounds. It is greatly involved in the improvement of seed germination performance in addition to the fruit yield [18]. Besides, SA is associated to the enhancement of plant tolerance to different abiotic stresses such as salinity and nutrient deficiency, including Fe [18–20]. Recent investigations [20] showed that the pretreatment of

seeds with SA and/or H_2O_2 enhanced tolerance to low Fe supply in *S. carnosa* plants, with the effect being partially dependent on the agent applied, genotype, and tissue. However, to our knowledge, the effect of SA seed priming on the phenolic c omposition and their related biological activities of *S. carnosa* under low Fe supply remain unknown. Thus, this study aims to shed light on the impact of SA seed priming on the phenolic profile in tissues of both varieties Sidi Khlif (SK) and Kalbia (KA) of the endemic Tunisian halophyte *S. carnosa*, as well as the antioxidant effectiveness of this species. Earlier investigation showed different physiological behavior of both varieties under low Fe supply [21]. In this work, we assess the effect of low Fe supply combined with SA seed priming on the (i) bivalent Fe and MDA contents (indicator of oxidative stress and Fe status, respectively), (ii) phenolic composition, and (iii) antioxidant activities.

2. Materials and Methods

2.1. Plant Growth Conditions and Treatments

Seeds of local halophytes, namely of *S. carnosa* varieties Sidi Khlif and Kalbia, were collected from two different saline biotopes, as described in our previous work [20]. After mechanic scarification, seeds were soaked 4h in SA solution with the following concentration: 0.25 mM. After that, germinated seedlings grown from unprimed and primed seeds (UPS and PS, respectively) were replicated in a half strength nutrient solution continually aerated for 7 days [20]. Similar-sized seedlings were then divided into blocks of 8 plants per 5 L pot, grown for 30 days in a full strength aerated nutrient solution. Finally, at the third leaf step, Sulla plants were separated in 2 groups (32 plants per group, 8 replicates per treatment) for 8 days, namely control (C: 50 µM Fe) and deficient (D: 1 µM Fe) plants, as shown in Table 1. The composition of solution medium was prepared as already described by Jelali et al. [20]. Fe was provided as Fe(III)–EDTA formula. The installation of hydroponic culture system was done in a growth chamber with the following characteristics: 24 °C/18 °C regime, 16/8 h for the day/night regime, photosynthetic photon flux density of 200 mmol m^{-2} s^{-1} and 70% for the relative humidity. At harvest, plants were separated into shoots (stems and leaves) and roots. Then, the fresh and dry weight (FW and DW, respectively) were determined.

Table 1. Treatments applied to *Sulla carnosa* seedlings.

	Nutrient Solution	Salicylic Acid (0.25 mM)	Fe (50 µM)
C	+	−	+
C+SA	+	+	+
D	+	−	−
D+SA	+	+	−

(−) absence or (+) presence of the solution. Control (C: 50 µM Fe); Deficient (D: 1 µM Fe); Salicylic acid (SA: 0.25 mM).

2.2. Chlorophylls

Chlorophyll was extracted from fresh leaf samples cut into discs in five milliliters of 80% acetone. The extraction was performed during 72 h in darkness at 4 °C. The extract absorbance was determined at 470, 649, and 665 nm. Pigment concentrations (mg g^{-1} FW) were calculated as indicated by Lichtenthaler [22] and using the following equations:

$$\text{Chlorophyll a (CHL a)} = 12.25A_{663} - 2.79A_{645}$$

$$\text{Chlorophyll b (CHL b)} = 21.50A_{645} - 5.10A_{663}$$

$$\text{Chlorophyll a + b (CHL a + b)} = 7.15A663 + 18.71A_{645}$$

A = absorbance at λ (nm).

2.3. HCl Extractible Iron (Fe^{2+})

Fe content was measured according to the procedure described by Wasli et al. [5]. *S. carnosa* samples were finely crushed in a standard agate crusher to avoid powder contamination by Fe traces. Then, 2 g of dry samples were digested with 15 mL HCl (1N) and vigorously shake. The absorbance was measured with an atomic absorption spectrophotometer (VARIAN 220 FS, Artarmon, Australia).

2.4. Stress Indicators Parameters

Levels of hydrogen peroxide (H_2O_2) were determined by measuring the titanium-peroxide complex, according to the procedure described by Jelali et al. [23], using 50 mg of fresh leaf/roots and mixing with cold pure acetone and centrifugation. Aliquots of 20% (*v*/*v*) $TiCl_4OH$ in concentrated HCl were combined with the supernatant to obtain a 4% (*v*/*v*) final concentration. After the supplement of NH_4OH (0.2 mL for each mL of sample) to precipitate the titanium-peroxide complex, DSS/DRS were centrifuged at 10,000 *g* for 5 min. The resulting pellet was washed five times in acetone and resuspended in 2N H_2SO_4. The absorbance of the solution was determined at 415 nm against a blank containing H_2O_2 instead of the sample extract.

The level of lipid peroxidation was measured as 2-thiobarbituric acid-reactive substances (mainly malondialdehyde (MDA), according to Jelali et al. [23], using 1 g of frozen fresh material that was homogenized in a mortar and pestle with 10 mL of (0.1%; p/v) trichloroacetic acid, followed by centrifugation. An aliquot of the upper liquid layer was added to 4 mL thiobarbituric acid (TBA) (0.5%; p/v) and, after centrifugation, absorbance of the supernatant was read (532 nm), subtracting nonspecific absorption (values at 600 nm). MDA concentration was calculated using its molar extinction coefficient (155 $mM^{-1} \cdot cm^{-1}$) with the formula:

$$\text{MDA (nm/g FW)} = [(OD_{532} - OD_{600})\text{*vs./0.155}] \times \text{FW}$$

2.5. Phenolic Compounds

The total phenolic and flavonoids contents in SK/KA plants were evaluated after extraction with methanol 80% (MeOH-E), as described by Wasli et al. [1]. Results were expressed as mg of gallic acid equivalent per g of dry plant weight (GAE/g DW) for TPC, and as mg (+)-catechin equivalent/g DW (mg CE/g DW) for TFC. In parallel, the individual phenolic compounds of the MeOH-E were identified by UHPLC-DAD-ESI/MS^n following the conditions previously described by Wasli et al. [5], using an Ultimate 3000 (Dionex Co., San Jose, CA, USA) apparatus equipped with an ultimate 3000 Diode Array Detector (Dionex Co., San Jose, CA, USA) and coupled to an ion trap MS equipped with an ESI source (Thermo LTQ XL, Thermo Scientific, San Jose, CA, USA). Quantification of the identified phenolic compounds was performed through the calibration curve of another compound from the same phenolic group and results were expressed in mg per g of dried extract.

2.6. In Vitro Antioxidant Capacities

2.6.1. Total Antioxidant Capacity (TAC)

Total antioxidant capacity was assessed according to Ben Mansour et al. [24]. Briefly, 100 µL of SK or KA extract was added to 1 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate), followed by incubation at 95 °C for 90 min and cooling. The absorbance was read at 695 nm and the results were expressed as mg gallic acid equivalent/g DW.

2.6.2. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Activity Assay

Antiradical capacity of SK and KA against DPPH radical was assessed according to Wasli et al. [1]. Briefly, 250 µL of methanolic solution of stable radical DPPH (0.2 mM) was added to 50 µL of increasing concentrations of SK or KA extract. After 30 min of incubation

at room temperature, the absorbance was read against a blank at 517 nm. DPPH scavenging ability was expressed as IC_{50} (mg/mL) which is the inhibiting concentration of 50% of the synthetic radical. The inhibition percentage (IP %) of DPPH radical was calculated using the following formula:

$$IP\ (\%) = [(A_{control} - A_{sample})/A_{control}] \times 100$$

2.6.3. Ferric Reducing Power Assay

The ferric reducing power was assessed by the altering of test solution from yellow to green due to the reduction of trivalent iron. Briefly, 1 mL of SK or KA varieties from different treatments with a concentration ranging from 0.1 to 3 mg/mL was mixed with 2.5 mL of Na_3PO_4 buffer (pH 6.6, 0.2 M) and 2.5 mL potassium ferricyanide $K_3Fe\ (CN)_6$; (1% *w*/*v*)]. After incubation in a water bath at 50 °C for 20 min, 2.5 mL of TCA (10%, *w*/*v*) were inserted followed by a vigorous centrifugation for 10 min at 650 g. At the final step, 2.5 mL of supernatant was blended with 2.5 mL of deionized water and 0.5 mL of $FeCl_3$ solution (0.1%, *w*/*v*), as described by Wasli et al. [1]. The absorbance was assessed at 700 nm against blank sample and ascorbic acid was used as a positive control. Results were expressed as EC_{50} values (mg/mL).

2.6.4. β-Carotene–Linoleic Acid Assay

β-carotene (0.2 mg) was dissolved in chloroform (1 mL), followed by the addition of linoleic acid (200 µL) and Tween 80 (0.2 mL) were inserted. After the evaporation of chloroform, oxygenated distilled water was added (50 mL) to the β-carotene/linoleic acid emulsion. Aliquots of this emulsion (3 mL) were transferred to test tubes containing 200 µL of extract (final concentration 1 mg/mL), followed by shaking and incubation at 50 °C in a water bath. The lipid peroxidation (LPO) inhibition was determined according to the following equation:

$$LPO\ inhibition\ (\%) = [(A_1 - A)/A_0 - A] \times 100$$

where A_1 = absorbance at T = 120 min A = absorbance of blank (sample without β-carotene) at T = 120 min A_0 = absorbance at T = 0 min. BHT was used as standard (R^2 = 0.98).

3. Results and Discussion

3.1. Fe Deficiency Impact on Physiological Traits

Since Fe is a cofactor of many redox proteins, it is considered as a noble element contributing to crucial metabolic function in plants [25]. Therefore, the exploration of metabolic changes observed under low Fe supply has recently gained great concern, especially with the novel priming approach adopted for the improvement of plants performance. In a previous investigation [20], deficient plants of *S. carnosa* primed with SA and/or H_2O_2 were shown to increase their root growth, in comparison with the unprimed ones, with a more evident effect observed in SK cultivar. This tolerant behavior was elucidated as a challenge of *S. carnosa* plants to improve Fe acquisition capacity through a more efficient exploration of the soil, in association with increased rhizosphere acidification and photosynthetic capacity. Similar behavior was previously described in other tolerant genotypes subjected to Fe constraint [5,25–27]. Nevertheless, no previous work was focused on the priming effect with SA on the secondary metabolism under low Fe supply, especially in *S. carnosa* plants. In this work, we investigate the potential relationship between SA and secondary metabolites generated under this environmental constraint.

As described Table 2, Fe deficiency decreased DW in shoots of *S. carnosa* plants grown from unprimed seedlings, when compared to the control (C). Nevertheless, the application of SA under control conditions (C+SA) or under low Fe supply (D+SA) repaired the harmful effect of Fe constraint via increasing the biomass production in both varieties. Indeed, shoot and root DW in addition to root/shoot ratio parameters were notably increased unprimed plants (C+SA) with respect to C plants. Regarding *S. carnosa* leaf photosynthetic

pigments, our results showed that Fe deficiency differently affected their levels (Table 2). Interestingly, the addition of SA (D+SA) treatment remediated the harmful impact of Fe stress for Chlorophyll a content, particularly in SK cultivar (+54% of the respective control D). However, a notable increase was noted for Chlorophyll b independent of cultivar, when compared to control plants.

Table 2. Physiological parameters of *Sulla carnosa* tissues (shoots and roots) in Kalbia and Sidi Khlif primed with SA and grown under different Fe concentrations for 8 days.

Varieties/Variables	C	C+SA	D	D+SA
Kalbia				
Shoot DW	165.2 ± 3 ^{C}	255.8 ± 3 ^{A}	94.6 ± 4 ^{D}	237.4 ± 3.0 ^{B}
Root DW	89.5 ± 3 ^{D}	149.5 ± 3.5 ^{B}	140.4 ± 2 ^{C}	159.7 ± 3.0 ^{A}
Plant DW	254.7 ± 5 ^{B}	405.3 ± 6 ^{A}	235 ± 4 ^{C}	397.1 ± 3 ^{A}
Root/Shoot DW	0.58 ± 0.04 ^{D}	0.84 ± 0.0 ^{A}	0.64 ± 0.0 ^{C}	0.70 ± 0.00 ^{B}
Fe^{2+} Shoot	1469 ± 44 ^{B}	1939 ± 26 ^{A}	816 ± 13 ^{C}	1484 ± 27 ^{B}
Fe^{2+} Root	1834 ± 36 ^{B}	1920 ± 6 ^{A}	1125 ± 18 ^{D}	1390 ± 52 ^{C}
CHL a	0.46 ± 0.13 ^{B}	0.77 ± 0.1 ^{A}	0.38 ± 0.0 ^{C}	0.36 ± 0.00 ^{C}
CHL b	0.26 ± 0.09 ^{B}	0.31 ± 0.03 ^{A}	0.11 ± 0.02 ^{D}	0.16 ± 0.03 ^{C}
CHL (a + b)	0.72 ± 0.20 ^{A}	1.08 ± 0.04 ^{B}	0.49 ± 0.02 ^{C}	0.52 ± 0.03 ^{C}
Sidi Khlif				
Shoot DW	164.5 ± 3.0 ^{C}	392 ± 11 ^{A}	96.5 ± 3.0 ^{D}	244 ± 2.0 ^{B}
Root DW	96.25 ± 3.2 ^{C}	163.3 ± 3 ^{B}	166.6 ± 7.0 ^{B}	176.6 ± 7.0 ^{A}
Whole plant DW	260.75 ± 2.5 ^{D}	475 ± 14 ^{A}	273.1 ± 9 ^{C}	411.6 ± 4.0 ^{B}
Root /Shoot	0.58 ± 0.1 ^{C}	1.83 ± 0.12 ^{A}	0.52 ± 0.0 ^{C}	0.68 ± 0.0 ^{B}
Fe^{2+} Shoot	2017 ± 77 ^{C}	2910 ± 53 ^{A}	871 ± 19 ^{D}	2247 ± 55 ^{B}
Fe^{2+} Root	1687 ± 27 ^{B}	2269 ± 79 ^{A}	1120 ± 8 ^{D}	1635 ± 27 ^{C}
CHL a	0.47 ± 0.02 ^{B}	0.79 ± 0 ^{A}	0.22 ± 0.0 ^{D}	0.34 ± 0.0 ^{C}
CHL b	0.23 ± 0.02 ^{A}	0.25 ± 0.01 ^{A}	0.07 ± 0.0 ^{C}	0.15 ± 0.01 ^{B}
CHL (a + b)	0.70 ± 0.11 ^{B}	1.04 ± 0.01 ^{A}	0.30 ± 0.02 ^{D}	0.49 ± 0.02 ^{C}

Plant DW, Root DW Shoot DW expressed as g/plant; chlorophylls contents expressed as mg/g FW; bivalent Fe (Fe^{2+}) content expressed as μg/g DW; CHL a: Chlorophyll a; CHL b: Chlorophyll b. C treatment (control: 50 μM Fe); D treatment (Deficient: 1 μM Fe); SA (Salicylic acid: 0.25 mM). Data are means of 6 replicates ± SE. Values in the same line with different superscripts are significantly different at $p < 0.05$.

Fe deficiency decreased the bivalent Fe content in *S. carnosa* tissues. In harmony with growth and total Fe status results [20], the reduction rate was more pronounced in KA compared to SK, particularly in shoots (ca. 56 and 44%, respectively), as compared to C plants. Interestingly, a notable rise of bivalent Fe content was noted in plants primed with SA. The degree of increase was more important in shoots when compared to roots (275 and 170% of the respective control), respectively in SK and KL, as presented in Table 2.

Thus, outcomes suggest that the different efficiency level noted in Fe deficient plants of *S. carnosa* primed with SA was not only related to the adequate modulation of the photosynthetic machinery and the acidification capacity [20], but also to the improved bivalent Fe remobilization from roots to shoots. This fact may be considered a novel *S. carnosa* approach based on the enhancement of root assimilation of this element that is not very accessible under Fe-scarce conditions, essentially in calcareous soils.

With this regard, recent investigations proved that the effectiveness of pretreatment seeds approach significantly depends on genotypic, seed physiology, and pretreatment agent type [16,26]. In addition, the bivalent Fe content decrease could be explained by the chlorophyll content reduction, as previously reported in several plants [16,26]. The critical function of Fe^{2+} in the regeneration of chlorophyll precursors was previously confirmed by Briat et al. [27].

3.2. Salicylic Acid Seed Priming Effect on Stress Indicator

As anticipated by Ksouri et al. [3], plants commonly react to oxidative stress via inducing the accumulation of secondary metabolites in order to mitigate different environmental

constraints. Indeed, phenolic compounds play an imperative function in the adaptive behavior to Fe deficiency due to their antioxidant and chelating properties [5]. Additionally, flavonoids have the ability to alter the membrane peroxidation via the alteration of the lipid filler arrangement and the reduction of its fluidity, which could delay the dispersion of free radicals and thus reducing damages of peroxidative reactions [28].

In order to correlate the Fe scarcity tolerance with defending mechanisms against oxidative stress, the levels of malondialdehyde (MDA) i.e., a marker of lipid peroxidation damage, were estimated in roots and shoots (Table 3). The results revealed that Fe deficiency increased MDA contents in tissues of both *S. carnosa* varieties, suggesting the occurrence of oxidative stress. In accordance with bivalent Fe content, changes in MDA levels were less evident in SK than in KA (126% and 119% of the respective control C), respectively, in leaves and roots. With this regard, Mittler [28] explained that membrane damage might be caused by high H_2O_2 levels, resulting in hydroxyl radical (OH^-) formation and consequently in lipid peroxidation. Previous investigations described incremented lipid peroxidation under Fe deficiency in other species, such as fennel [1] and pea [23], in addition to other abiotic stresses [28]. Saharkhiz and Goudarzi [29] reported that foliar spray with SA decreased MDA content in peppermint plants under water stress, exhibiting an important role in maintaining the membrane structure by preventing damage caused by this constraint. Low membrane lipid peroxidation in wheat plants grown under water stress and supplemented with SA (0.5 mM) was also reported by Kang et al. [30].

Table 3. Malonylaldehyde (MDA, µmol g/FW), Total phenolic compounds (TPC, mg GAE/g DW) and Total Flavonoids (TFC, mg CE/g DW) contents of *Sulla carnosa* cv. Kalbia (KA) and Sidi Khlif and (SK), primed with salicylic acid (SA) and grown under different Fe concentrations for 8 days.

Varieties/Variables	C	C+SA	D	D+SA
Kalbia				
MDA (L)	3.12 ± 0.76^{C}	1.69 ± 0.40^{D}	5.65 ± 0.18^{A}	4.23 ± 0.68^{B}
MDA(R)	3.29 ± 0.28^{B}	1.93 ± 0.54^{C}	5.56 ± 0.09^{A}	3.22 ± 0.95^{B}
TPC	7.19 ± 0.13^{A}	6.93 ± 0.22^{A}	3.49 ± 0.29^{B}	2.71 ± 0.19^{C}
TFC	3.26 ± 0.28^{A}	3.04 ± 0.21^{A}	1.53 ± 0.21^{B}	1.15 ± 0.15^{C}
Sidi Khlif				
MDA (L)	4.19 ± 0.17^{C}	3.26 ± 0.20^{D}	5.32 ± 0.28^{A}	4.83 ± 0.18^{B}
MDA (R)	6.13 ± 0.28^{B}	2.74 ± 0.18^{C}	7.30 ± 0.05^{A}	3.38 ± 0.79^{C}
TPC	4.46 ± 0.09^{C}	5.87 ± 0.37^{A}	4.13 ± 0.27^{C}	5.06 ± 0.13^{B}
TFC	2.51 ± 0.28^{C}	4.41 ± 0.12^{A}	2.15 ± 0.27^{C}	3.38 ± 0.15^{B}

C treatment (control: 50 µM Fe); D treatment (Deficient: 1 µM Fe); SA (Salicylic acid: 0.25 mM). L—leaves; R—roots. Data are means of 4 replicates ± SE. Means in the same line followed by distinct letters are significantly different at $p \leq 0.05$.

3.3. Effect of SA Seed Priming on the Phenolics Composition

Antioxidant and chelating properties of phenolic compounds are essential in the adaptive behavior to Fe deficiency [1]. Therefore, total phenolic (TPC) and flavonoid contents (TFC) were studied in hydromethanolic extracts of both control and treated *S. carnosa* plants. As summarized in Table 3, the level of phenolics varied significantly in both varieties, being superior in KA (7.19 ± 0.13 mg GAE/gDW for TPC and 3.26 ± 0.28 mg CE/g DW for TFC, respectively) compared to SK (4.46 ± 0.09 mg GAE/gDW for TPC and 2.15 ± 0.07 mg CE/g DW for TFC, respectively). Under D conditions, a significant decrease of phenolic pools was observed in KA (ca. 51 and 53%, respectively for TPC and TFC, compared to the control C), while the contents of such metabolites remained unchanged in SK. The two varieties also reacted differently to SA treatments: SK raised the contents of TPC and TFC either under Fe sufficiency or deficiency conditions, whereas almost no changes occurred in KA plants.

UHPLC-DAD-ESI-MS^n analysis was performed for further clarification of phytochemicals in *S. canosa* plants with different treatments (C, C+SA, D, and D+SA). The corresponding UV-Vis and MS^n spectral data of the detected peaks are summarized in

Table 4, of which twelve phenolic compounds were identified. The most representative was eluted in peak 16 at RT 12.1 min, exhibiting a λ_{max} at 221 and 280 nm, and a $[M-H]^-$ at m/z 289, generating a base fragment ion at m/z 245 by the loss of 44 Da (equivalent to a CO_2), that in turn fragmented to m/z 179 and 151 in MS^3 experiment, being thus assigned to epi-catechin as its described by Sun et al. [31]. Besides, the extracts contained other flavonoids as minor components: apigenin-6,8-di-*C*-glucoside (peak 14, λ_{max} at 256, 354 nm, $[M-H]^-$ at m/z 593]; rutin ($[M-H]^-$ at m/z 609→301 and luteolin-7-*O*-rutinose ($[M-H]^-$ at m/z 593→285 eluted in peaks 15 and 17, respectively). Hydroxycinnamic acids (HCA) were also major phenolic constituents in *S. carnosa* leaves of both varieties. Overall, in plants grown under optimal Fe supply (control), caffeic acid (peak 12, $[M-H]^-$ at m/z 179) and its derivatives, including four caffeoylglucaric acids isomers (eluted in peaks 4, 5, 6, and 8, of $[M-H]^-$ at m/z 371) and two caffeoylquinic acid isomers (peaks 7 and 11, $[M-H]^-$ at m/z 353), were detected, together with a ferulic acid derivative, namely ferulolylglucaric acid (peak 13 $[M-H]^-$ at m/z 385 → 209, 353, 191).

Table 4. Identification of UHPLC eluting fractions by UHPLC-DAD-MS^n of *Sulla carnosa* hydromethanolic extracts.

Peak	Rt (min)	λmax	$[M-H]^-$	Main MS Fragments	Probable Compound
1	1.4	224	133	155, 73	Malic acid
2	1.8	258	191	127, 173, 111, 85, 93	Quinic acid
3	1.8	258	191	111, 173, 67	Citric acid
4	2.6	290sh, 324	371	209, 353, 191	Caffeoylglucaric acid (isom 1)
5	3.3	295sh, 325	371	209, 353, 191	Caffeoylglucaric acid (isom 2)
6	4.2	295sh, 325	371	209, 353, 191	Caffeoylglucaric acid (isom 3)
7	5.0	295sh, 325	353	191, 179, 173	Caffeoylquinic acid (isom 1)
8	5.5	289sh, 323	371	209, 353, 191	Caffeoylglucaric acid (isom 4)
9	5.8	279	209	191, 193	Glucaric acid
10	6.0	279	323	119, 113, 101, 143, 179 161, 131, 149, 95	Unknown
11	7.8	297sh, 322	353	191, 179, 161, 135	Caffeoylquinic acid (isom 2)
12	8.7	298sh, 327	179	135, 179	Caffeic acid
13	8.9	221, 326	385	191, 367, 209, 281	Feruloylglucaric acid
14	9.7	234, 273, 324	593	473, 503, 353, 575,383	Apigenin-6,8-di-C-glucoside
15	11.6	256, 356	609	301	Rutin
16	12.1	221, 280	289	245, 205, 203	Epicatechin
17	12.4	268sh, 341	593	285	Luteolin-7-*O*-rutinose
18	15.8	ND	327	309, 291, 239, 171	Unknown
19	16.7	ND	267	193, 135	Unknown
20	16.9	ND	327	309, 291, 293, 171	Unknown
21	20.7	ND	823	171, 193	Unknown
22	20.5	247, 285	987	ND	Unknown
23	21.2	254, 281	831	ND	Unknown

ND—Not detected; sh—UV shoulder.

The phenolic components in plants grown under deficient conditions (D, D+SA) corresponded to those grown in the presence of Fe (C, C+SA), regardless significant variations on their levels (Table 5). In accordance with the results relative to TPC and TFC (Table 4), the gathered data indicated that, under complete medium primed with SA (C+SA), *S. carnosa* SK increased greatly the biosynthesis of HCA, compared to C plants. Moreover, when grown under Fe deficiency, SK did not show a significant change of its phenolic compound levels compared to C, contrarily to KA that was characterized by a significant decrease of HCA and flavonoid amount (32% and 16%, respectively in D versus C, respectively). Under this deficiency, soaking with SA did not reverse the levels of HCA in KA, but again, a slight improvement of phenolic contents was observed in SK cultivar (11% and 19% for HCA and flavonoids, respectively).

Table 5. Contents of individual phenolic compounds (mg/g DW) in *Sulla carnosa* leaves of Kalbia (KA) and Sidi Khlif varieties, primed with salicylic acid (SA) and grown under different Fe concentrations for 8 days.

	C Kalbia	C+SA Kalbia	D Kalbia	D+SA Kalbia	C Sidi Khlif	C+SA Sidi Khlif	D Sidi Khlif	D+SA Sidi Khlif
Hydroxicynnamic acids								
CGA_{isom1}	2.57 ± 0.14^{A}	2.47 ± 0.07^{B}	1.07 ± 0.32^{F}	1.38 ± 0.05^{D}	1.23 ± 0.17^{E}	1.85 ± 0.14^{C}	0.91 ± 0.02	1.37 ± 0.04^{D}
CGA_{isom2}	5.21 ± 1.09^{B}	5.75 ± 0.88^{A}	3.56 ± 0.09^{D}	2.91 ± 0.03^{F}	2.09 ± 0.3^{H}	3.95 ± 0.07^{C}	2.83 ± 0.11^{G}	3.24 ± 0.32^{E}
CGA_{isom3}	4.24 ± 0.09^{C}	5.23 ± 0.16^{A}	3.74 ± 0.25^{E}	3.95 ± 0.13^{D}	3.26 ± 0.2^{F}	4.79 ± 0.21^{B}	3.10 ± 0.01^{G}	3.95 ± 0.01^{D}
CGA_{isom4}	6.44 ± 1.56^{A}	5.88 ± 0.33^{C}	4.37 ± 0.08^{E}	4.02 ± 0.41^{F}	4.65 ± 1.1^{D}	6.13 ± 0.09^{B}	4.68 ± 0.01^{D}	4.70 ± 0.08^{D}
CQA_{isom1}	1.37 ± 0.14^{D}	1.03 ± 0.08^{H}	1.29 ± 0.41^{E}	1.25 ± 0.12^{F}	1.82 ± 0.1^{B}	2.55 ± 0.31^{A}	1.70 ± 0.04^{C}	1.20 ± 0.10^{G}
CQA_{isom2}	1.49 ± 0.00^{A}	0.97 ± 0.19^{B}	0.56 ± 0.03^{D}	0.50 ± 0.74^{E}	0.42 ± 0.06^{F}	0.85 ± 0.02^{C}	0.40 ± 0.01^{F}	0.59 ± 0.00^{D}
CA	0.75 ± 0.04^{A}	0.69 ± 0.00^{B}	0.24 ± 0.011^{F}	0.23 ± 0.01^{F}	0.47 ± 0.03^{D}	0.72 ± 0.07^{A}	0.36 ± 0.09^{E}	0.56 ± 0.01^{C}
$\sum$	22.28 ± 3.06^{A}	22.35 ± 1.73^{A}	15.06 ± 1.20^{D}	14.39 ± 1.49^{E}	14.25 ± 2.14^{E}	21.23 ± 0.92^{B}	14.30 ± 0.29^{E}	15.86 ± 0.56^{C}
Flavonoids								
R	3.32 ± 0.87^{A}	3.13 ± 0.24^{B}	1.80 ± 0.09^{E}	2.67 ± 0.09^{C}	1.49 ± 0.18^{G}	1.85 ± 0.45^{D}	1.29 ± 0.13^{H}	1.66 ± 0.14^{F}
EpiC	15.84 ± 3.17^{B}	15.33 ± 1.41^{B}	14.87 ± 2.65^{C}	12.80 ± 0.98^{D}	12.54 ± 0.54^{D}	16.50 ± 1.47^{A}	12.23 ± 2.33^{E}	14.67 ± 1.88^{C}
LutR	2.11 ± 0.05^{A}	2.56 ± 0.35^{A}	1.24 ± 0.12^{F}	1.88 ± 0.12^{C}	1.87 ± 0.23^{C}	2.02 ± 0.11^{B}	1.59 ± 0.09^{E}	1.65 ± 0.03^{D}
$\sum$	21.27 ± 4.09	21.02 ± 2.00^{A}	17.91 ± 2.86^{C}	17.35 ± 1.19^{D}	15.90 ± 0.59^{E}	20.37 ± 2.03^{B}	15.11 ± 2.55^{F}	17.98 ± 2.05^{C}

C treatment (C (control: 50 µM Fe); D treatment (Deficient: 1 µM Fe); SA (Salicylic acid: 0.25 mM); CA—caffeic acid; CGA—Caffeoylglucaric acid; CQA—Caffeoylquinic acid; isom—Isomer; EpiC—epicatechin; LutR—Luteolin-*O*-rutinose; R—rutin. Means in the same line followed by distinct letters are significantly different at $p \leq 0.05$.

Phenolics were described as the major classes of organic ligands displayed by strategy I plants under Fe deficient conditions. In this respect, caffeic acid and ferulic acid were previously proposed to sustain Fe reduction from ferrihydrite [32]. Other phenolics such as quercetin, myricetin and rutin were also implicated in the increase of Fe uptake, mainly through the stimulation of Fe reduction mechanism [33,34]. Alternatively, Wasli et al. [5] supposed that the allocation of additional biomass from shoot to root organ via the reduction of lignin production by hydroxycinnamic acids is affected by Fe uptake under bicarbonate induced Fe deficiency.

Phenolic compounds as stress markers are in general stimulated under stressful environment. It is believed that soluble phenolic compounds act as scavengers of ROS and membrane stabilizers during abiotic stress [1,35]. Considered together, the stimulation of phenolic compounds in Fe deficient plants primed with SA could be considered as a strategy adopted by *S. carnosa* to counteract the oxidative stress and indirectly facilitate root Fe assimilation (described in Table 3), which is an input function in the adaptation of strategy I plants to Fe deficiency. In this context, Jin et al. [35] proposed that phenolic metabolites might boost the apoplastic Fe exploitation and consequently improve Fe nutrition in shoots. In fact, phenolics induced by Fe deficiency play a crucial function in the regulation of Fe assimilation through improved accumulation in the root apoplast. Perron and Brumaghim [36] later confirmed that phenolic compounds function as antioxidants by chelating metal or scavenging ROS for defense against oxidative damage.

In this context, SA applied as foliar spray in peppermint plants under water stress increased the total phenolic contents, compared to control plants [29], with this accumulation being dependent on the severity of the stress and the SA concentration. Moreover, in the same plant species, Figueroa Pérez et al. [37] reported that total levels of flavonoids were increased in relation to control by approximately 93%, 100%, and 56%, when treated with 0.5, 1, and 2 mM SA, respectively. The same effect was reported in ginger plants as well, with an increment of TPC of approximately 20% being registered after treatment with 10 µM SA [38].

In our study, the priming with SA for control or deficiency conditions did not change the contents of TP and TPC in KA cultivar. Yet, SK primed seeds showed an improvement in the contents of such compounds, under control or Fe shortage conditions (Tables 3 and 5). It is possible that this effect in SK cultivar may be related to the decrease of lignin biosynthesis, since phenolic compounds, such as hydroxycinnamic acids, are considered as lignin precursors, whereas lignification reduction has been recommended to be an adjustment reaction to Fe deficiency [5]. On the other hand, hydroxycinnamic acids are considered as

the main bioactive secondary metabolites in plants serving as scavengers of ROS under adverse environmental conditions (such as wounding, drought, metal toxicity, and nutrient deprivation) [39,40].

Plants often respond to environmental stresses with an increase in the endogenous SA level [41]. In fact, SA is a promising compound for the reduction of stress sensitivity in the practical agriculture. Kováčik et al. [42] pointed out that SA treatment in chamomile caused different behavior which varies from growth-promoting (at 50 µM) to growth-inhibiting effects (at 250 mM). In addition, the high SA dose resulted in the rise of the phenylalanine-ammonia-lyase (PAL) activity, followed by an increase in the accumulation of soluble phenolic compounds and lignin. In another work [43], the authors attested that SA treatment may stimulate the increase of phenolic pools, which affects the tolerance to stress provoked by different agents. In this context, a study in wheat showed that exogenous (hydroponic) SA application enhanced the expression of genes involved in the flavonoid metabolism [44]. Notably, SA is a phenylpropanoid derivative and primarily functions as a defense compound in plant–biotic interactions [45].

3.4. Effect of SA Seed Priming on Antioxidants Activities

S. Carnosa leaf extracts of both varieties were investigated for their antioxidant abilities through distinct in vitro methods, namely total antioxidant activity (TAC), DPPH•, and β-carotene and FRAP assays, allowing to evaluate their scavenging ability towards distinct radicals, as well as the ability to reduce Fe^{3+} to Fe^{2+} and inhibiting the bleaching of the antioxidant pigment β-carotene.

As shown in Table 6, maximal antioxidant capacities under control conditions (+50 µM Fe) were observed in KA (as indicated by inferior IC_{50} and EC_{50} values), a fact that is probably linked to its high TPC and TFC contents, compared to SK (Table 3).

Table 6. Antioxidant activities of *Sulla carnosa* cv. Kalbia (KA) and Sidi Khlif and (SK), primed with salicylic acid (SA) and grown under different Fe concentrations for 8 days.

Varieties/Variables	C	C+SA	D	D+SA
Kalbia				
TAC (mg GAE/g DW)	12.89 ± 1.12^{B}	14.33 ± 3.15^{A}	10.37 ± 0.63^{C}	7.54 ± 0.44^{E}
DPPH (IC_{50} mg/mL)	2.00 ± 0.03^{C}	1.66 ± 0.14^{D}	2.98 ± 0.24^{B}	3.87 ± 0.56^{A}
FRAP (EC_{50} mg/mL)	1.76 ± 0.11^{C}	1.50 ± 0.01^{D}	2.12 ± 0.11^{B}	3.54 ± 1.13^{A}
β-carotene (IC_{50} mg/mL)	2.87 ± 0.18^{B}	2.11 ± 0.19^{D}	2.10 ± 0.09^{D}	3.06 ± 0.34^{A}
Sidi Khlif				
TAC (mg GAE/g DW)	9.35 ± 0.12^{D}	14.25 ± 2.33^{A}	12.87 ± 1.76^{B}	12.99 ± 2.44^{B}
DPPH (IC_{50} mg/mL)	2.82 ± 0.06^{B}	1.31 ± 0.07^{E}	1.98 ± 0.01^{C}	1.93 ± 0.09^{C}
FRAP (EC_{50} mg/mL)	2.15 ± 0.24^{B}	1.27 ± 0.17^{E}	1.66 ± 0.45^{D}	1.57 ± 0.14^{D}
β-carotene (IC_{50} mg/mL)	3.12 ± 0.02^{A}	2.14 ± 0.01^{D}	2.45 ± 0.13^{C}	3.01 ± 0.03^{A}

TAC—total antioxidant capacity, DPPH—2,2-Diphenyl-1-picrylhydrazyl radical scavenging activity; FRAP—Ferric reducing antioxidant power; β-carotene—β-Carotene-linoleic acid assay; C treatment (control: 50 µM Fe); D treatment (Deficient: 1 µM Fe); SA (Salicylic acid: 0.25 mM). Data are means of 3 replicates ± SE. Means in the same line followed by similar letters are significantly different at $p \leq 0.05$.

Under complete medium soaked with SA (C+SA), an enhancement in all antioxidant activities was depicted for both varieties predominantly for SK (Table 6). Indeed, results from TAC, DPPH•, β-carotene, and FRAP were improved by about 23%, 56%, 58%, and 31%, respectively, in SK cultivar versus 10%, 17%, 15%, and 26% in KA. Whereas Fe shortage reduced the antioxidant activity in KA, against an increase noted in SK. In turn, priming seeds with SA under Fe deficiency condition (D+SA) was shown to effectively enhance the common antioxidant activities, both in SK and KA plants.

As presented in Table 7, the dependence on antioxidant activity, obtained from various assays, in relation to TPC/TFC had a linear correlation in SK cultivar between the values of TAC (r = 0.99 and 0.96) and the IC_{50} values of scavenging activity, DPPH (r = –0.76 and r= –0.80), β-carotene linoleic acid model system (r = –0.91 and–0.96), and the EC_{50}

value of FRAP (r = –0.88 and–0.97). In the same line, a strong correlation was detected in KA in the case of TAC (r = 0.83 and 0.92), DPPH$^{\bullet}$ quenching activity (r = –0.86 for TPC and r = –0.94 for TFC and β-carotene linoleic acid model system (r = –0.76 and –0.83), respectively. Indeed, antioxidant capacity and phenolic content were positively correlated because phenolic compounds directly affected antioxidant activity [5]. Nevertheless, a weak link connecting TPC (or TFC) and antioxidant activity was noted for FRAP assay in KL, with r values under 0.5. Likewise, a strong linear correlation was mainly established between caffeic acid derivatives and individual flavonoids, particularly in SK cultivar.

Table 7. Correlation coefficients between total and individual phenolic compound amounts and antioxidant activities.

Variables	TPC	TFC	TAC	DPPH	β-Carotene	FRAP
Kalbia						
TPC (mg GAE/g DW)	1	0.96	0.83	−0.86	−0.76	−0.01
TFC (mg GAE/g DW)	0.96	1	0.92	−0.94	−0.83	−0.16
TAC (mg GAE/g DW)	0.83	0.92	1	−0.99	−0.95	−0.51
DPPH (IC_{50} mg/mL)	−0.86	−0.94	−0.99	1	0.94	0.46
β-carotene (IC_{50} mg/mL)	−0.76	−0.83	−0.95	0.94	1	0.6478
FRAP (EC_{50} mg/mL)	−0.01	−0.16	−0.51	0.46	0.64	1
CGA_{isom1} (mg/gDW)	0.95	0.85	0.71	−0.75	−0.72	0.01
CGA_{isom2} mg/gDW)	0.92	0.82	0.69	−0.73	−0.73	−0.02
CGA_{isom3} mg/gDW)	0.94	0.85	0.73	−0.77	−0.76	−0.05
CGA_{isom4} mg/gDW)	0.97	0.91	0.70	−0.75	−0.62	0.19
CQA_{isom1} mg/gDW)	0.80	0.89	0.99	−0.99	−0.97	−0.58
CQA_{isom2} mg/gDW)	−0.32	−0.16	−0.20	0.21	0.45	0.27
CA (mg/gDW)	0.80	0.64	0.33	−0.3968	−0.26	0.54
R (mg/gDW)	0.78	0.6107	0.44	−0.4927	−0.54	0.11
EpiC (mg/gDW)	0.72	0.5883	0.53	−0.5658	−0.68	−0.18
LutR (mg/gDW)	0.69	0.5485	0.49	−0.5225	−0.65	−0.16
Sidi Khlif						
TPC (mg GAE/g DW)	1	0.94	0.99	−0.76	−0.91	−0.88
TFC (mg GAE/g DW)	0.94	1	0.96	−0.80	−0.96	−0.97
TAC (mg GAE/g DW)	0.99	0.96	1	−0.82	−0.95	−0.89
DPPH (IC_{50} mg/mL)	−0.76	−0.80	−0.82	1	0.94	0.66
β-carotene (IC_{50} mg/mL)	−0.91	−0.96	−0.95	0.94	1	0.87
FRAP (EC_{50} mg/mL)	−0.88	−0.97	−0.89	0.66	0.87	1
CGA_{isom1} (mg/gDW)	0.96	0.82	0.94	−0.71	−0.82	−0.73
CGA_{isom2} (mg/gDW)	0.90	0.89	0.93	−0.96	−0.97	−0.77
CGA_{isom3} (mg/gDW)	0.90	0.89	0.93	−0.96	−0.97	−0.77
CGA_{isom4} (mg/gDW)	0.99	0.97	0.99	−0.81	−0.95	−0.92
CQA_{isom1} (mg/gDW)	0.92	0.82	0.88	−0.46	−0.70	−0.83
CQA_{isom2} (mg/gDW)	0.94	0.94	0.93	−0.60	−0.83	−0.96
CA (mg/gDW)	0.90	0.70	0.85	−0.55	−0.68	−0.62
R (mg/gDW)	0.85	0.73	0.86	−0.87	−0.84	−0.56
EpiC (mg/gDW)	0.91	0.99	0.94	−0.84	−0.97	−0.96
LutR (mg/gDW)	0.95	0.95	0.97	−0.92	−0.99	−0.85

Data represents Pearson Correlation Coefficient R; TPC—total phenolic compound; TFC—total flavonoid content; TAC—total antioxidant activity; DPPH—2,2-Diphenyl-1-picrylhydrazyl radical scavenging activity; FRAP—FRAP reducing power potential; β-carotene—β-Carotene-linoleic acid assay. CA—caffeic acid; CGA—Caffeoylglucaric acid; CQA—Caffeoylquinic acid; isom—Isomer; EpiC—epicatechin; LutR—Luteolin-*O*-rutinose; R—rutin.

In this regard, Pérez-Tortosa et al. [46] showed that SA applied at 10 µM concentration on culture media of *Thymus membranaceus* shoots resulted in the increased accumulation of some phenolic compounds, essentially the rosmarinic acid rate, which in turn enhanced the antioxidant capacity, proving the high tolerance of such species. The same authors reported a high association between soluble flavanols and antioxidant activity, as evaluated by the DPPH (r > 0.5) method. The flavanols antioxidant activity has been ascribed to

the existence of a catechol group on the B ring, which chelates redox-active metals and scavenges free radicals, specifically superoxide radical, singlet oxygen, and lipophilic alkyl peroxyl radical [47]. It is imperative to point out that the overall antioxidant capacity shown by complex extracts in these assays was probably influenced by the amount of additive, as well as synergistic and/or antagonistic impacts of the individual compounds. Therefore, although it is not simple to indicate the antioxidant capacity shown by plant extracts toward a specific phenolic class, it is probable that the oxidation of flavanols to Pas observed in SA-treated shoots might provide a backup defense system against oxidative stress, thus contributing to the attenuation of lipid peroxidation.

4. Conclusions

Outcomes showed that the application of SA under deficient and/or control conditions improved tolerance to Fe shortage of *S. carnosa* plants with different levels between both varieties. The better efficiency of this species could be related to the better modulation of secondary metabolite biosynthesis, namely phenolic compounds, in addition to the improvement of antioxidant activity, as assessed by five different test systems, suggesting a sufficient defense against oxidative damage. Evaluation of the impact of SA on the behavior of secondary plant metabolism with economic benefits, especially for the use of pastures, such as *S. carnosa*, could provide a basis for their rational planting in alkaline soil with an innovative program aimed at improving the production and yield of selected new effective Fe genotypes. These findings should encourage the application of SA by farmers as an efficient biological tool in calcareous soils in mass crop intensification programs for this pastoral species.

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