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# L-DOPA Trends in Different Tissues at Early Stages of *Vicia faba* Growth: Effect of Tyrosine Treatment

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**Featured Application:** The potential applications of this work are in human health and in plant pest control. In humans: L-DOPA is the first-line treatment for Parkinson's disease. In plants, L-DOPA has insecticidal and allelochemical properties.

**Abstract:** The nonprotein amino acid Levo-3,4-dihydroxyphenylalanine (L-DOPA) has insecticidal, allelochemical, and antiparkinsonian effects. The aim of this research was to assess L-DOPA content in different tissues of *Vicia faba* (cv. Super Agua Dulce), and to verify if treatment with the phenolic amino acid L-4-hydroxyphenylalanine (tyrosine) had an effect on such content. Under light germination, control and tyrosine-treated early seedling stages of *V. faba* were studied and L-DOPA was quantified spectrophotometrically (Arnow's method) and by high-performance thin-layer chromatography (HPTLC), as well. Additionally, tyrosinase (TYROX) and guaiacol peroxidase (GPX) activities (considered markers of a phenolic compounds metabolism) were quantified as germination proceeded. Different organs (roots, sprouts, and seeds) and different developmental stages were considered. Steady high L-DOPA concentrations were found in untreated sprouts and roots compared to seeds, as time progressed. While TYROX activity was not detected in these experiments, GPX had diverse trends. In control tissues, GPX increased in seed tissue as germination progressed, whereas in roots and sprouts, a decreasing GPX activity was observed. Tyrosine exposure decreased L-DOPA content, and decreased or did not change GPX activity (depending on the organ). Both Arnow's and HPTLC methods were consistent in terms of tendencies, except for the scarce contents found in seeds, in which HPTLC was more sensitive. The richest source of L-DOPA was found in shoots (untreated), reaching as high as 125 mg g<sup>-1</sup> DW (12% in DW) (the highest content reported in fava bean seedlings until now), whereas the smallest L-DOPA content was found in seeds. The importance of light germination conditions is discussed in terms of L-DOPA yield and from a physiological perspective. It is concluded that *V. faba* (cv. Super Agua Dulce) shoots are a good source of L-DOPA and that tyrosine addition (0.55 mM) decreases L-DOPA content in actively growing tissues (shoots and roots).

**Keywords:** *Vicia faba*; L-DOPA; HPTLC; tyrosine; enzymes

## 1. Introduction

Levo 3,4-dihydroxyphenylalanine (L-DOPA) therapy is necessary for Parkinson's disease for its powerful antiparkinsonian effect [1]. L-DOPA is a nonprotein amino acid with biological activity, found in animals and in some plants [2]. It is the precursor of catecholamine type neurotransmitters (dopamine, norepinephrine, and epinephrine), being involved in melanin synthesis as well [3]. Lately, L-DOPA has drawn much attention in the field of plants for its acknowledged defensive

role against insects [4]. In addition, it is considered an allelochemical, i.e., a compound released into the environment affecting the growth and development of neighboring plants [1,5,6].

L-DOPA has raised particular interest in legumes, particularly in the Fabaceae family, as reports describe a relatively high content in such plants [7,8]. Additionally, for Parkinson's patients, fava bean consumption would be even better than the drug in terms of motor performance and side effects [1]. Moreover, carbidopa (a peripheral inhibitor of the enzyme DOPA decarboxylase) has been found in fava bean as well [9]. Thus, different tissues of fava bean have been analyzed for L-DOPA, and as much as 6.0–6.75% dry weight (DW) (60–67.5 mg g<sup>-1</sup> DW) has been found in young pods of cultivars Diana and Alameda, respectively [10], and contents in whole seedlings in the order of 0.2% DW (2 mg g<sup>-1</sup> DW) [11].

L-DOPA would be produced via the oxidation of the phenolic amino acid L-4-hydroxyphenylalanine (tyrosine) by the enzyme tyrosinase (tyrosine oxidase: TYROX) [12]. In turn, tyrosine comes in plants from the shikimic acid pathway and TYROX would be involved in L-DOPA synthesis in non-Cariophyllales plants [13]. TYROX is involved in other polyphenolics and melanin synthesis, as well [6]. L-DOPA is a melanin biosynthetic precursor (present in plant and animal tissues). In general, TYROX are multicopper monophenol monooxygenases (EC 1.14.18.1), which use oxygen as cosubstrate to catalyze the o-hydroxylation of phenols (i.e., tyrosine to L-DOPA step); and the subsequent oxidation of the formed o-diphenols to the corresponding o-quinones as well. Thus, using the aromatic amino acid L-tyrosine as substrate, the ultimate tyrosinase-catalyzed reaction product is dopaquinone [13]. This o-quinone can undergo chemically spontaneous and also enzymatic reactions, which in turn can further convert it into melanin. Therefore, TYROX is considered a key enzyme for melanin synthesis [14] and has also been associated with stress responses to the environment, as melanin plays a survival function [15].

L-DOPA is also a precursor of phenylpropanoids in plants; the phenylpropanoid pathway is an important metabolic route, as it synthesizes phenolic compounds, including lignin [16]. Indeed, in plants, TYROX plays an important role in modifying and hardening the protective exterior layer of, for instance, seed envelopes, and as an agent against invasive organisms [16].

In plants, a common early response to environmental changes is the antioxidant enzymatic pathway, with roles in signal transduction and pathogen defense, producing protective biomolecules such as melanin and lignin [17]. GPX (Guaiacol peroxidase, (EC 1.11.1.7)) is a heme-containing protein that oxidizes certain phenolic substrates at the expense of H<sub>2</sub>O<sub>2</sub> [18]. GPX is considered a marker phenolic compounds metabolism and antioxidant response. Indeed, GPX has a role in the biosynthesis of lignin, the defense against biotic stresses, decomposition of indole-3-acetic acid (IAA), among others [19,20], and is widely accepted as a 'stress enzyme'. Amongst the various antioxidant enzymes, GPX can be considered one of the key ones, since both of its extra and intracellular forms participate in the breakdown of H<sub>2</sub>O<sub>2</sub> [15,20].

In an effort to stimulate L-DOPA production in fava bean, Randhir et al. [21] studied L-DOPA content in seedlings, primed with oregano extract, lactoferrin, and a fish protein hydrolysate (dark germination). They found a positive effect of priming seeds with all those former elicitors, finding a fast response in lactoferrin primed seedlings, with a L-DOPA content of 3.5% DW approx (data transformed from the author using 90% water content). On the other hand, Raghavendra et al. [12] reported that in *Mucuna pruriens* suspended tissue cultures, the phenolic amino acid L-tyrosine increased L-DOPA production, finding that the highest concentration of L-DOPA obtainable was 9.47 ± 0.70% DW (40.13-fold increase). Although this latter study achieved a 40-fold L-DOPA content increase by adding tyrosine to cell tissue cultures of *M. pruriens*, no information is available in terms of tyrosine effects on DOPA content in legume germinating seeds. Moreover, there is no evidence on whether such an interaction involves the tyrosine related enzymes formerly described.

The aim of this research is to verify if tyrosine treatment has an effect on L-DOPA content in different tissues at the early developmental stages of *Vicia faba*. Concomitant monitoring of TYROX and GPX activities, as related markers of phenolic metabolism, was recorded as well. The plants'

response to the treatment in terms of growth rate was measured too. In addition, the L-DOPA content was assessed and compared using two different analytical methods.

## 2. Materials and Methods

Solvents were of HPLC grade. USP standard of L-DOPA and Guaiacol 98% were obtained from Sigma-Aldrich (St. Louis, MO, USA). Ethanol, H<sub>2</sub>O<sub>2</sub> 30% p.a., Dichloromethane 99.9%, Isopropanol 99.8%, Acetic acid 96%, water LiChrosolv<sup>®</sup> LC-MS grade, and HPTLC plates silica gel were obtained from Merck (Darmstadt, Germany), and formic acid 98% from Fluka (Buchs, Switzerland). Polyvinylpyrrolidone (PVP) was obtained from Calbiochem (Selangor, Malaysia).

### 2.1. Experimental Setup

*V. faba* seeds (Super Agua Dulce variety, Agroflora<sup>®</sup>) and coconut fiber (Cocomix, BioBizz<sup>®</sup>) were obtained at local market suppliers. Control and tyrosine-treated seeds were primed for 48 h in distilled water and tyrosine (0.55 mM), respectively. They were watered each 3–4 days with 50 mL per tray, with distilled water and tyrosine solution (0.55 mM), respectively. The experiment was performed in triplicate. Each tray contained 300 g of coconut fiber and 20 seeds (sawn at surface, under natural light conditions in the laboratory).

### 2.2. Plant Extracts

Three different organs were considered: Roots, sprouts and seeds. Their respective enzymatic activities, L-DOPA content, protein content, and dry mass (lyophilized) were followed in each seedling developmental stage: 3–4 days (A); 8–9 days (B) or 2–4 leaves (C, 15 days approx.).

The activity of enzymes described below was determined spectrophotometrically from the accumulation of the respective product using a Shimadzu UV-1240 spectrophotometer and presented as mmol min<sup>-1</sup> g<sup>-1</sup> dry weight. One gram of each tissue was manually ground with a mortar in 3–6 mL of cold extraction buffer (0.05 M potassium phosphate buffer pH 6.5, 0.5% polyvinylpyrrolidone). Samples were centrifuged at 11,000 × *g* for 10 min and stored on ice. The resulting supernatant was the extract used in all enzyme assays.

### 2.3. Enzyme Assays

#### 2.3.1. TYROX

Dopachrome production in each organ enzyme extract was followed spectrophotometrically at 475 nm (modified from Kampmann et al. [22]). Assays were conducted at 25 °C, in a final volume of 1 mL reaction mixture that was prepared as follows: 50 µL of fava bean organ extract was added to 950 µL of 10 mM L-DOPA (in 0.05 M phosphate buffer, pH 6.0) to start the reaction. Enzyme activity was determined from the linear portion of the curve, using a Dopachrome  $\epsilon_{475} = 3.6 \text{ mM}^{-1} \text{ cm}^{-1}$ ; proper blanks were considered and a positive control for tyrosine oxidase (*Beta vulgaris* roots) was run in parallel as well.

#### 2.3.2. GPX

Tetraguaiacol was followed at 470 nm. The assay was performed as follows (modified from Jebara et al. [23]): Briefly, to a final volume of 1 mL (25 °C), a reaction mixture consisting of 930 µL 0.05 M phosphate buffer (pH 7.0), 50 mM guaiacol, 50 µL of fava bean organ extract, and 20 µL 0.2 mM H<sub>2</sub>O<sub>2</sub> were added. Enzyme activity was calculated at a fixed time interval of 0.5 min, determined from the linear portion of the curve, using a tetraguaiacol  $\epsilon_{470} = 26.6 \text{ mM}^{-1} \text{ cm}^{-1}$  [23,24], (corresponding blanks were run in parallel).

#### 2.4. Growth Rate Record

Growth rates were determined using the respective photographic records with a reference scale in centimeters. These measurements were performed with the software ImageJ (1.49v, USA) [25] for three parameters: Root length, sprout length, and number of roots. The root and sprout length were determined from the longest root and the longest shoot apices to the cotyledon stalk, respectively. Regarding the number of roots, each apex of primary and secondary roots was labeled by a dot and then quantified.

#### 2.5. L-DOPA Content in *V. faba*

Plant organ extracts (obtained as the extracts for enzymatic activity) were used to determine the L-DOPA content using Arnow's method [26]. L-DOPA was further quantified using ultraviolet coupled high-performance thin-layer chromatography HPTLC–UV. Briefly, lyophilized samples of each organ were ground to a fine powder (Bertin Instruments, Precellys 24 homogenizer, ceramic balls, for 30 s at 5000 rpm) and extracted at room temperature, as follows. Each sample (50–200 mg) was extracted with 1:1 formic acid–ethanol (for 2 h, 2 mL) under continuous agitation (120 rpm). Afterwards, samples were centrifuged for 10 min at  $10,000\times g$  and supernatants were saved. The resulting pellets were resuspended in new 1:1 formic acid-ethanol (2 h, 2 mL, 120 rpm), followed by the centrifugation step (10 min at  $11,000\times g$ ). Thus, pellets were repeatedly (4 times) subjected to the same extraction cycle. Finally, pellets were extracted for the last time over a longer period of time in 1:1 formic acid-ethanol (2 mL, overnight, and 120 rpm). The five supernatants involved in the overall process were pooled and finally centrifuged (10 min  $10,000\times g$ ). These collected supernatants were used as the respective fava bean extracts loaded on the HPTLC plates for further analysis.

Fava bean extract samples and standard solutions were applied by means of Automatic TLC Sampler 4 (ATS 4) from Planar Chromatography Manager CAMAG (Muttensz, Switzerland), with the following settings for 18 tracks per plate: Band length 6.0 mm, track distance 10.5 mm, applied volume range loaded of 2–20  $\mu\text{L}$ , dosage speed  $100\text{ nL s}^{-1}$ , and application position x-axis 10.0 mm and y-axis 10.0 mm each. Chromatography was carried out in a  $20\times 10\text{ cm}$  twin trough chamber (CAMAG) using 10 mL of dichloromethane:isopropanol:acetic acid:water as 2:2:1:1 ( $v/v/v/v$ ) as mobile phase, up to a migration distance of 80 mm. After development, the plate was dried in a stream of warm air for 1 min. Detection was performed with TLC Scanner 3 (CAMAG) in UV-absorption mode at 282 nm with a slit dimension of  $5.0\text{ mm}\times 0.2\text{ mm}$  and a scanning speed of  $20\text{ mm s}^{-1}$ . All instruments were controlled via software platform winCats 1.4.6 (CAMAG).

#### 2.6. Statistical Analysis

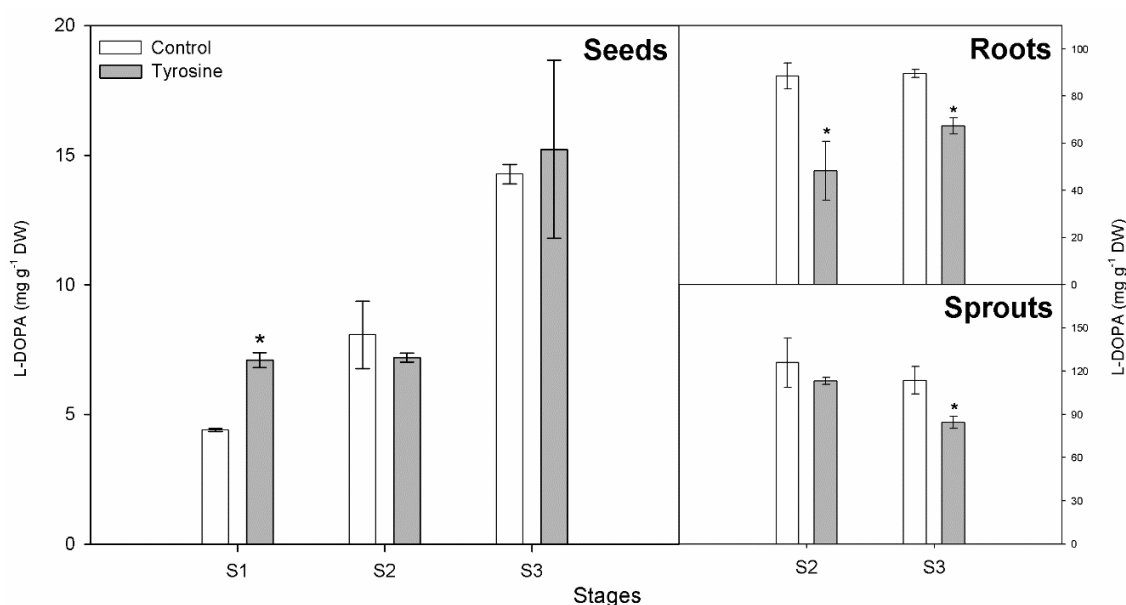
Differences between control and tyrosine treatments in terms of their L-DOPA content, GPX activity, and growth records were assessed by Tukey's test ( $P < 0.05$ ). These analyses were performed with the InfoStat/L software package (FCA-UNC, Argentina). A Pearson correlation analysis was performed to assess the relationship between the HPTLC and Arnow's method for L-DOPA quantification, using Statistica 7.0 (Stat Soft Inc., Tulsa, OK, USA).

### 3. Results and Discussion

#### 3.1. L-DOPA Content

With regard to L-DOPA concentration in control tissues: Although low contents were found in seeds, an increasing tendency in time could be observed until stage S3 (2–4 leaves emerged, 15 days approx), reaching concentrations up to  $14.2\pm 0.38\text{ mg g}^{-1}\text{ DW}$  ( $1.42\pm 0.03\%\text{ DW}$ ) (Figure 1). Steady high concentrations were found in sprouts and roots as time progressed. Indeed, concentrations as high as  $125.87\pm 17.18\text{ mg g}^{-1}\text{ DW}$  ( $12.5\pm 1.7\%\text{ DW}$ ) were observed in sprouts (S2 stage: 8–9 days), i.e., 10 times higher than in seeds, sprouts being the richest sources of L-DOPA found (Figure 1). This is in accordance with Goyoaga [11], who observed similar trends as germination took place in a *V. faba*

embryo axis with a concentration range of  $20.3 \text{ mg g}^{-1} \text{ DW}$  (at day 0) up to approximately  $80 \text{ mg g}^{-1} \text{ DW}$  at 9 days, in light germination as well.



**Figure 1.** Effect of tyrosine treatment on Levo-3,4-dihydroxyphenylalanine (L-DOPA) content in *Vicia faba* seeds, roots, and sprouts at the early stages of development: S1: 3–4 days, S2: 8–9 days, and S3: 2–4 leaves. The bars indicate  $\pm$  standard error ( $n = 3$ ). \* indicates significant differences between controls and tyrosine treatments according to Tukey ( $P < 0.05$ ).

The L-DOPA content decreased with tyrosine treatment at almost all stages of roots and sprouts. In seeds, no significant differences in L-DOPA concentrations were detected at almost all stages, except for stage S1 (3–4 days) (Figure 1), in which a slight increase in such content was found.

It must be noted that light exposure during germination seems to be an important factor in the accumulation of L-DOPA, since Shetty et al. [27], Randhir et al. [21], and Vattem et al. [28] describe a totally different trend, i.e., decreasing L-DOPA content at the early stages of *V. faba* being germinated in darkness (values in the order of  $4 \text{ mg g}^{-1}$  to  $1.5 \text{ mg g}^{-1} \text{ FW}$  in from day 0 to day 8, respectively). Indeed, they report L-DOPA contents that decrease gradually as plant growth proceeds, which suggests that L-DOPA is only synthesized, or even that it could be possibly only imported, at the early developmental stages, in dark germination [28,29]. On the contrary, increasing DOPA contents were observed in light germinating faba bean ( $4$  to  $7 \text{ mg g}^{-1} \text{ FW}$  from day 12 to day 16, respectively) [30]. Moreover, Wichers et al. [31] reported an apparent light effect in L-DOPA content in *Mucuna pruriens* cell liquid culture (a threefold increase as compared with dark condition). In that sense, our results agree with those of Goyoaga et al. [11] and Wichers et al. [31] in that they both worked with legumes in light environments, finding, in Goyoaga's study, a very high L-DOPA content in germinating faba bean harvested 9 days after imbibition ( $80 \text{ mg g}^{-1} \text{ DW}$ ) [11] and, in Wichers et al. [31], comparing a light versus a dark condition, finding more than three times L-DOPA under light culturing experiments with suspended *Mucuna* cells in liquid medium.

From a physiological perspective, light enhanced L-DOPA production is consistent with developmental needs, i.e., more L-DOPA content needed as the plantule emerges above ground as an herbivory preventive strategy. Therefore, if L-DOPA maximal yield potential is sought, dark germination should be avoided in the experimental set ups.

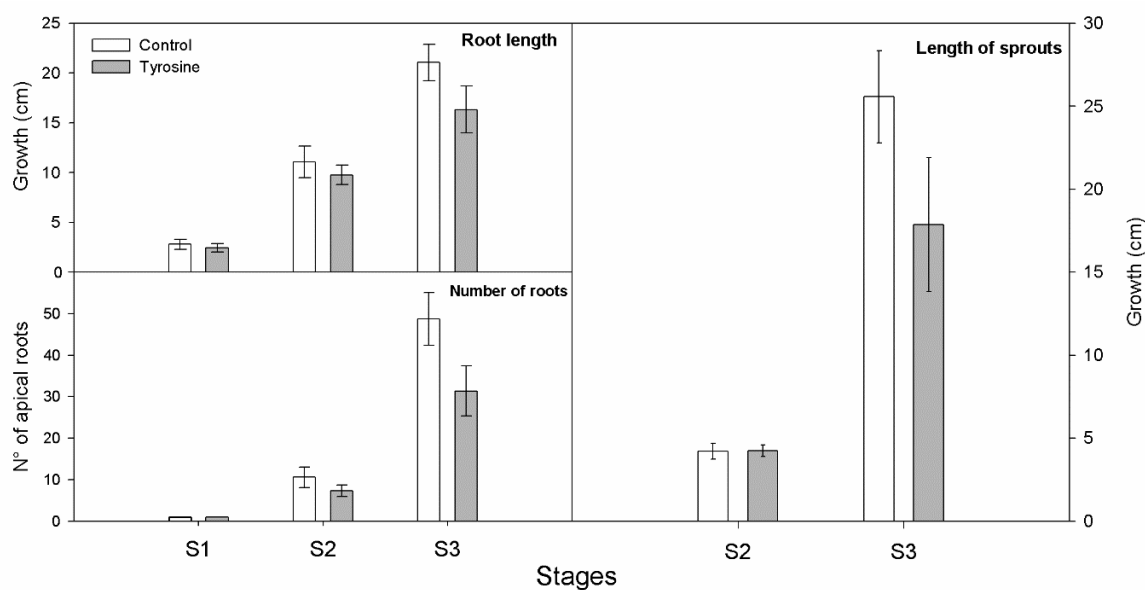
Recently, in a long-term greenhouse light germinating study, the highest L-DOPA concentration found in *V. faba* seedlings harvested after 15 days of germination was  $13.3 \text{ mg g}^{-1} \text{ DW}$  [32]. It must be noted that the study weighed the whole seedling and did not separate individual tissues at that first stage of plant development, which could be one of the causes of a lower value than the former ones.

Additionally, the same study determined L-DOPA content in leaf, weighing just that specific organ tissue, finding  $10.5 \text{ mg g}^{-1} \text{ DW}$ . In other legumes belonging to the *Mucuna* gender, relatively high L-DOPA has been detected in different organs, such as leaves and seeds, containing as much as 1% DW and 4–7% DW L-DOPA, respectively [33–35]. Ramya and Thakur [36] compiled L-DOPA content in other genera, such as *Canavalia*, *Glycine*, *Vigna*, and others, with relatively low concentrations (0.2–1.9% DW).

Regarding tyrosine availability in plants, recent findings suggest that enhanced tyrosine supply would enhance betalain synthesis (other DOPA-derived secondary metabolite) in betalain-producing Caryophyllales [13]. Our results do not prove any stimulatory growth effect (Figure 2), nor an enhanced L-DOPA content. On the contrary, we found that tyrosine treatment decreased DOPA content, and this could be related to tyrosine degradation as an energy source (catabolic pathway). Tyrosine-free levels can vary inside plants, and its catabolism has the highest amino acid energy yield feeding the tricarboxilic acid cycle [37]. Additionally, it must be noted that another potential route for tyrosine catabolism has been suggested via L-DOPA catabolism by Ellis et al. [38]. Other possible final destinations of tyrosine have to be considered as well, such as protein synthesis and other molecules, because tyrosine is the precursor of many other diverse metabolites, so its final destiny can be molecules such as isoquinoline alkaloids, lignin, and tyramine, among others [13].

As stated earlier, environmental stress is associated with both enzyme activity and secondary metabolite accumulation. Faba bean is a Nitrogen fixing plant and tyrosine contains Nitrogen; hence, there is a possibility that seedlings could have experienced stress by Nitrogen supplementation. Our results cannot support such an association. Indeed, considering GPX as stress marker (see Section 3.3), no increment in this enzyme activity or in the L-DOPA content was found (see Figure 1). This latter finding is in agreement with a recent report that describes that a wide range of an inorganic fertilizer supplementation (calcium ammonium nitrate) in faba bean does not reflect any significant difference in L-DOPA content [32].

Studies in which tyrosine is provided to cultured cells (of other plant species) have shown an increment in L-DOPA content. Apart from the study of Raghavendra et al. [12] performed in *Mucuna* tissue culture, a high biotransformation rate of tyrosine to L-DOPA in liquid medium cultured cells of *Portulaca grandiflora* has been reported as well [39].



**Figure 2.** Effect of tyrosine treatment on root length, number of roots, and length of sprouts in *V. faba* at the early stages of development: S1: 3–4 days, S2: 8–9 days, and S3: 2–4 leaves. Bars indicate  $\pm$  standard error ( $n = 3$ ). No significant differences between controls and tyrosine treatments were found, Tukey ( $P < 0.05$ ).

### 3.2. Comparison of Methods for L-DOPA Determination

Both Arnow’s and HPTLC methods for L-DOPA determination were consistent in terms of tendencies and magnitudes found, except for the scarce content found in seeds. In the latter, the HPTLC procedure was more sensitive than Arnow’s (3–5-fold).

Although HPTLC consistently yielded a higher content of L-DOPA than Arnow’s (Table 1) in all *V. faba* organs analyzed (probably due to the more efficient extraction employed), Arnow’s method for L-DOPA determination seems to be a valuable tool in screening L-DOPA content. Indeed, it is a simple, inexpensive, and robust method for an evaluation of this compound. Hence, if a relatively high content of L-DOPA is detected in faba bean by simple mortar extraction and Arnow’s method, a much higher concentration should be expected with more exhaustive extractions and sensitive techniques, such as HPTLC.

**Table 1.** L-DOPA content (mg g<sup>-1</sup> dry matter) in different *V. faba* tissues.

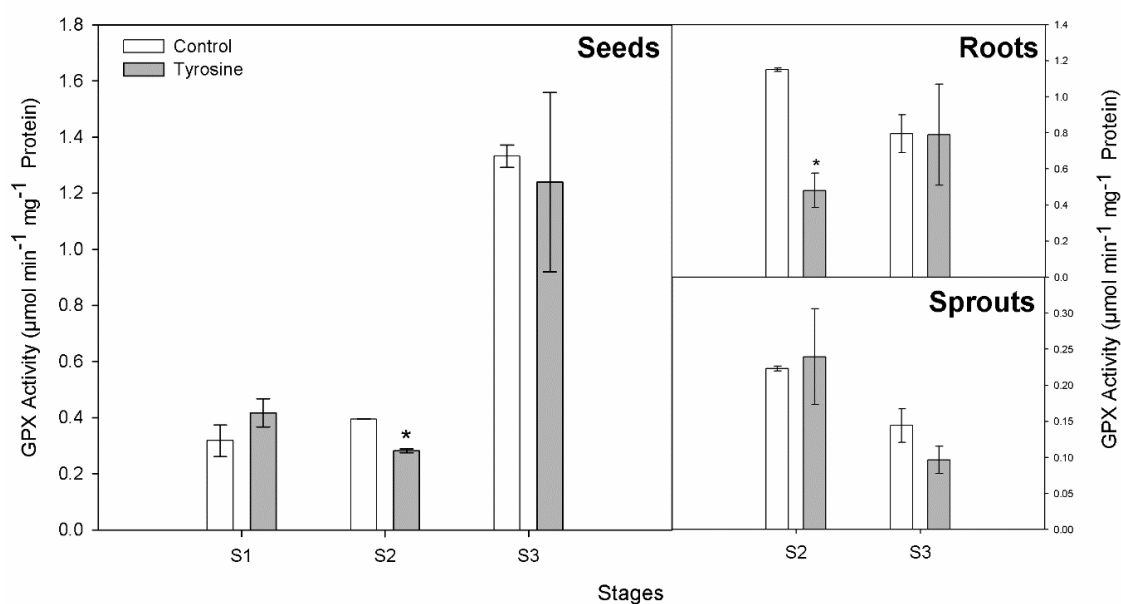
Stages	Arnow			HPTLC		
	Seed	Root	Sprout	Seed	Root	Sprout
S1	0.90 ± 0.11	*	*	4.40 ± 0.06	*	*
S2	1.90 ± 0.16	69.43 ± 3.05	81.25 ± 2.91	8.07 ± 1.30	88.50 ± 5.54	125.87 ± 17.18
S3	5.69 ± 0.26	69.45 ± 5.33	89.28 ± 2.06	14.27 ± 0.38	89.67 ± 1.76	113.60 ± 9.53

\* Nonexistent organ.

In seeds, the correlation between L-DOPA content measured by HPTLC and Arnow’s (Vis-spectrophotometry), respectively, was estimated by the Pearson correlation method. In this case, a good correlation between the resulting measurements was observed: 0.96 ( $y = 0.4787x - 1.43$   $R^2 = 0.93$ ), Arnow’s L-DOPA content being 31% of the respective HPTLC values.

### 3.3. GPX and TYROX Activities

In control tissues, GPX only increased in the seed tissue as germination progressed, until stage S3 (2–3 leaves) (Figure 3). This in agreement with Shetty et al. [7,27]. In roots and sprouts, a decreasing GPX activity was observed (Figure 3).



**Figure 3.** Effect of tyrosine treatment on guaiacol peroxidase (GPX) activity in *V. faba* seeds, roots, and sprouts at the early stages of development: S1: 3–4 days, S2: 8–9 days, and S3: 2–4 leaves. The bars indicate ± standard error ( $n = 3$ ). “\*” indicates significant differences between controls and tyrosine treatments according to Tukey ( $P < 0.05$ ).

GPX elicitation has been described as an early indicator of environmental changes in plants [15]. Indeed, the elevation of GPX activity has been reported as the only more general response to stimuli, such as metal exposure in *V. faba* [40]. Interestingly, Kabbadj [41] found that different cultivars of fava bean had different GPX responses to water deficit, and only the Reina Mora cultivar exhibited an increase in GPX activity.

Our results do not reflect any increased GPX activity with tyrosine treatment. On the contrary, GPX activity decreased or did not vary, depending on the organ (Figure 3). GPX has been proposed as a phenolic marker metabolism; thus, precursors derived from shikimate route could be used by GPX for phenolic polymerization in structural development processes (germination) [29]. In our experiment, tyrosine treatment implies abundant phenolics, suggesting no need of increment in GPX activity. TYROX activity was not detected in all our experiments. The positive control for tyrosine oxidase (*Beta vulgaris* roots) was run in parallel in all samples and yielded proper activities  $0.6 \text{ mmol min}^{-1} \text{ g}^{-1} \text{ DW}$ .

#### 4. Conclusions

Overall, it can be stated that to the best of our knowledge, the occurrence of such concentrated L-DOPA content in specific organ tissues has never been reported. Sprouts of *V. faba* (var. Super Agua Dulce) have the highest L-DOPA content detected until now  $125 \text{ mg g}^{-1} \text{ DW}$  (12% DW). It can be also concluded that tyrosine exposure (0.55 mM) does not enhance L-DOPA content and that the high L-DOPA content found in the latest stage of growth studied (sprouts) does not seem to be related to GPX or TYROX activities. In terms of L-DOPA quantification, both Arnow's and HPTLC methods were consistent in terms of tendencies and magnitudes found, except for the scarce contents found in seeds, in which HPTLC was more sensitive.

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**Conflicts of Interest:** Claudia Oviedo-Silva, Mharyn Elso-Freudenderga, and Mario Aranda-Bustos declare that they have no conflict of interest.

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