Biotin supplementation alters root system architecture and development in *Arabidopsis thaliana*

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Abstract: Biotin is a member of the vitamin B-complex family that acts as a cofactor of carboxylases and is essential in the metabolism of all organisms. In addition to its essential metabolic functions, biotin has been suggested to play a critical role in regulating gene expression in plants and animals. However, all studies in plants have been conducted under biotin deficiency. Therefore, we hypothesized that exogenously supplied biotin also exerts non-enzymological functions similar to those reported in animals, regulating root growth and development in *Arabidopsis thaliana*. To test this hypothesis, we evaluated the effects of the biotin supply on seedlings and analyzed the root architecture. Biotin supplementation inhibited root elongation and promoted lateral root formation in a concentration-dependent manner. Inhibited primary root elongation was correlated with decreased expression of the cell cycle genes *CycB1;1:uidA* and cell expansion gene *pAtExp7::uidA*, and depended on the concentration of biotin. Viability tests with *AtHisH2B:YFP* showed that the meristem was depleted. However, biotin supplementation did not affect the concentration of chlorophyll but had a slight inhibitory effect on foliage growth and increased the production of reactive oxygen species (ROS) at the apex of primary roots. Our study provides the first evidence of functions of biotin supplementation in plants in addition to its catalytic role as an enzyme cofactor, likely advancing our understanding of the biological functions of biotin.

Keywords: *Arabidopsis thaliana*, biotin, development, growth, root architecture

Abbreviations: ACC, acetyl-CoA carboxylase; DHE, dihydroethidium; LRN, lateral root number; MCC, β -methylcrotonyl-CoA carboxylase; MS, Murashige and Skoog Medium; PI, propidium iodide; PR, primary root meristem; PYF, protein yellow fluorescent; ROS, reactive oxygen species; X-Gluc, 5-bromide-4-chloride-3-indol- β -D-glucuronide

Introduction

The function of vitamins is of great interest not only in animals but also in plants because they are essential for its metabolism (Ayala et al. 2017). Biotin is a water-soluble vitamin of the B-complex and is essential in all organisms because it is the cofactor of carboxylases, which have an essential function in the metabolism of carbohydrates, amino acids and lipids (Alban et al. 2001; Nikolau et al. 2003). Furthermore, in organisms that synthesize biotin (bacteria, plants and some fungi) (Roje 2007), these enzymes also have important functions in CO₂ fixation (Berg et al. 2010), acetyl-CoA production (Khomyakova et al. 2011), complex fatty acids biosynthesis (Gago et al. 2011), terpenoid metabolism (Forster and Jendrossek 2010) and in the use of urea as a nitrogen source (Navarathna et al. 2010).

There are several reports of the function of biotin as a regulator of genetic expression in addition to its catalytic function as a coenzyme for carboxylases in

bacteria, animals and plants (Cronan 1989, Rodriguez and Zemleni 2003, Zemleni 2005, Li et al. 2012, Maruyama et al. 2012). Chen et al. (2003) reported that biotin regulates the expression of methylcrotonyl-CoA carboxylase via a mechanism that is independent of its function as a prosthetic group. Additionally, experiments with the lethal mutant of *Arabidopsis thaliana bio1-1* have demonstrated its participation in growth and development; these works reported that embryogenesis was arrested in the globular form because these mutant embryos were incapable of synthesizing biotin, and they were rescued by addition of biotin and desthiobiotin (Schneider et al. 1989). Homozygotic mutants for the biosynthesis of biotin in *A. thaliana* (*bio-1*, *bio-2* and *bio-3*) are lethal for embryos (Muralla et al. 2008, Pommerrenig et al. 2013), while mutant *bio4-1* plants exhibit a massive accumulation of hydrogen peroxide and constitutive up-regulation of genes that are diagnostic for defense and reactive oxygen species signaling (Li et al. 2012). These results suggest that this vitamin participates in processes other than classical carboxylation reactions, such as growth and development.

Although genetic evidence has demonstrated that biotin biosynthesis is important for the activation of defense signaling, programmed cell death (Li et al. 2012) and regulation of 3-methylcrotonyl-coenzyme A carboxylase expression in plants (Chen et al. 2003), the effects of biotin application on root growth or lateral root formation are unknown. Therefore, we examined the effect of biotin at supraphysiological concentrations on the growth and development of the radicular architecture of *Arabidopsis thaliana*, via several mechanisms that are independent of its role as a cofactor in the carboxylation reaction. In addition to supporting the plant, the root system is essential for the absorption of nutrients, minerals, and water. Thus, the development of the root and its architecture and morphology can be modified by environmental factors, such as the temperature, humidity and availability of nutrients (Aceves et al. 2016).

Our data indicated that biotin had an inhibiting effect on primary root growth, as evidenced by the termination of root apical meristem growth. Biotin promoted the production of lateral roots in seedlings due to meristematic cell differentiation. However, the vitamin did not modify cell viability or the concentration of chlorophyll, but provoked a slight decrease in foliage development at high concentrations. We further observed an increase in the production of reactive oxygen species (ROS) at the apex of the primary root by decreasing the rate of cell division. Our findings underline the importance

of biotin as a bioactive vitamin that acts as signal triggering molecular mechanisms that modify cell division and development processes within the root. These observations suggest that in addition to its catalytic role as an enzyme cofactor, biotin has a role modifying the configuration of the root system architecture.

Materials and Methods

Plant materials and growth conditions

Arabidopsis thaliana ecotype Columbia (Col-0), mutant lines *pAtExp7::uidA* (Cho and Cosgrove 2002), *CycB1;1:uidA* (Colon et al. 1999) and *AtHisH2B:YFP* (Boisnard et al. 2001) were used. Seeds were sterilized with 95% (v/v) ethanol for 5 min and chloride solution (Sodium hypochlorite, NaClO) 15% (v/v) for bleaching for 7 min. After four washes with sterile distilled water, seeds were germinated and grown on agar plates containing $0.2 \times$ Murashige and Skoog (MS) medium (4.3 g L^{-1} salts for a $1 \times$ concentration of medium; we used 0.9 g L^{-1} for $0.2 \times$ MS) (Murashige and Skoog, 1962) either with or without D-biotin (250, 500, and 1000 μM) in a 1:1 mixture of deionized water: DMSO, supplemented with sucrose 0.6% (w/v) and phytagar (Phytotechnology) 1% (w/v). The pH was adjusted to 7.0 before addition of the agar. As reported in several studies examining the rescue of the biotin mutant phenotype (Li et al. 2012, Muralla et al. 2008, Chen et al. 2003, Pommerrenig et al. 2013), of biotin concentration range from 250 μM to 1000 μM was used. Biotin and MS medium were purchased from Sigma-Aldrich (catalog M5524 and 58-85-5, respectively). The plates with germinating seeds were incubated in a plant growth chamber (Percival Scientific AR-95L) under continuous fluorescent lights with a photoperiod of 16 h of light and 8 h of dark, a light intensity of $150 \mu\text{mol m}^{-2} \text{ s}^{-1}$, and a temperature of 22°C. Plates were placed vertically to avoid root penetration in the medium.

Analysis of the root architecture

Arabidopsis thaliana root system and primary root length (PRL) meristem integrity were quantified with a ruler and stereoscopic microscope (Leica MZ6) in 30 seedlings from 2 independent growth Petri dishes. The lateral root number (LRN) was quantified 13 days after germination by counting the LRN present in the primary root per seedling from the apex to the transition zone between the root and stem with an optical microscope (Leica EZ4 D; Germany). Lateral root density (LRD) was calculated by dividing the LRN value between the PRL value for each analyzed

seedling (cm^{-1}), according to Ayala et al. (2017) and Raya et al. (2014). Images were counted at X30 magnification with a SAMSUNG SCC 131-A (Samsung, South Korea) digital color camera adapted to the microscope and processed with Zeiss AXIO VISION 4AC software (Carl Zeiss, Pleasanton, USA).

Determination of foliage weight

At 13 day after germination, stalks of 20 seedlings were cut regardless of the treatment. The foliage was measured on a Sartorius ISO 9001 ultra-precision analytical weighing scale (Sartorius, Germany).

Histochemical analysis

To determine the activity of the reporter gene *Uida* (GUS), transgenic lines *pAtExp7::uidA* (Cho and Cosgrove 2002) and *CycB1;1::uidA* (Colon et al. 1999) were incubated in solutions containing 0.1% X-Gluc (5-bromide-4-chloride-3-indol- β -D-glucuronide) in sodium phosphate buffer (NaH_2PO_4 and Na_2HPO_4 , 0.1 M, pH 7) with 2 mM potassium ferrocyanide and 2 mM potassium ferricyanide, overnight at 37°C. The plants were clarified and fixed according to Ayala et al. (2017). Briefly, the plants were cleared and fixed with 0.24 N HCl in 20% (v/v) methanol and incubated for 60 min at 62°C and posteriori the solution was substituted for 7% NaOH (w/v) in 60% (v/v) ethanol for 20 min at room temperature. The plants were dehydrated with ethanol treatments at 40%, 20% and 10% (v/v) for a 24h period each and fixed in 50% glycerol (v/v). The treated roots were placed on slides and sealed with commercial nail varnish. Ten primary root of each experimental group was observed and photographed under a microscope (Leica MZ6/L2) at a magnification of X 40. To quantify the expression of *CycB1;1::uidA*, 10 roots were photographed under a magnification of X 40 using a microscope (Leica MZ6/L2; Germany) with a 5 mm FMA050 camera (Leica). The cells expressing *CycB1;1::uidA* in the apical meristem were counted.

Cell viability

Transgenic seedlings expressing *pAtHisH2B::YFP* (Boisnard et al. 2001) were incubated with propidium iodide (PI; 10 mg/mL) on a slide for 1 min. Seedlings were rinsed in water and mounted in 50% (v/v) glycerol on microscope slides. Seedlings were analyzed individually at wavelengths of 568 nm for excitation and 585-610 nm for emission; these conditions permit the use of PI and yellow fluorescent protein (YFP) (488 nm excitation and

505-550 nm emission). The stained tissues were photographed using a confocal microscope (Olympus FV1000; Olympus Corp., Japan).

Detection of ROS

General ROS were determined in *Arabidopsis thaliana* seedlings. Briefly, seedlings were incubated for 30 min in the dark with a solution of the fluorophore label (H2DCF-DA, Sigma-Aldrich, USA) (10 μM) and washed three times for 5 min with fresh buffer (Gomes et al. 2005). Fluorescence signals from at least 30 treated and control seedlings were detected using a confocal microscope (Olympus FV1000, Olympus Corp., Japan).

Chlorophyll quantification

The total chlorophyll content was estimated according to Hanjagi and Singh (2017). Briefly, 50-100 mg fresh leaf samples were incubated for 4 h in DMSO at 65°C in the dark. The samples were immediately cooled at room temperature, and the wavelengths specific to chlorophyll were recorded at 663 nm (α -chlorophyll) and 645 nm (β -chlorophyll) using DMSO as blank. The results are expressed as mg g^{-1} fresh weight.

Statistical analysis

Values are expressed as the means $\pm$ standard error of the mean (SEM). Experimental data were analyzed statistically with the SigmaPlot® 11.0 program using ANOVA, followed by univariate and multivariate analyses with Tukey's posthoc test to evaluate the differences in growth and root development responses. Differences between groups were considered significant at $P < 0.05$. The measurements were repeated in at least 30 plants from two independent experiments.

Results

Biotin modifies *Arabidopsis* root growth and architecture

We evaluated the biotin effect on the root development and architecture of *Arabidopsis thaliana* at 13 days after germination and clearly showed the root architecture reprogramming in response to biotin. Seedlings showed concentration-dependent primary root growth inhibition in medium supplied with biotin (Fig. 1A) and an increased number of lateral roots in the primary root (Fig. 1B). An analysis of the lateral roots showed that biotin increased this lateral root density by four-fold (Fig.

Fig. 1. Effects of biotin on *Arabidopsis thaliana* root architecture. Wildtype (Col-0) seedlings were grown for 13 days under increasing biotin concentrations on agar-solidified $0.2 \times \text{MS}$ medium vertically oriented agar plates. A) Primary root length, B) lateral root number, C) lateral root density. Values shown represent the mean $\pm$ SEM of 30 seedlings. Representative photographs of agar plates with seedlings supplemented with (d) 0, (e) 250 (f) 500 and (g) 1000 μM biotin. Different letters indicate statistical differences at $P < 0.05$. Scale bar = 1 cm.

Fig. 2. Effect of biotin on cell elongation primary root. Transgenic *Arabidopsis thaliana* seedlings expressing *AtExp7::uidA* were germinated, and seedlings were grown for 13 days on agar-solidified $0.2 \times \text{MS}$ medium. A) Primary roots were stained for GUS activity and showed expression of the *CycB1:uidA* gene construct. B) Analysis of meristematic zone length. Data represent the mean $\pm$ SEM ($n=30$). Different letters indicate statistical differences at $P < 0.05$. Scale bar = 0.1 mm.

Fig. 3. Effect of biotin on cell division in root meristems. *Arabidopsis thaliana* seeds harboring the *CycB1;1:uidA* gene construct were germinated and seedlings were grown for 13 days on agar-solidified $0.2 \times$ MS medium. A) Photographs show representative individuals from 30 GUS-stained seedlings. B) Number of GUS-positive spots per root meristem. Values shown represent the mean of 30 seedlings $\pm$ SEM. Different letters indicate statistical differences at $P < 0.05$.

Fig. 4. Effect of biotin on total fresh weight of foliage. *Arabidopsis thaliana* (Col-0) seedlings were germinated and grown in $0.2 \times$ MS medium for 13 days with increasing biotin concentrations. A) Photographs are representative individuals of at least 30 *Arabidopsis thaliana* plants. B) Foliage fresh weight was recorded. Data represent the mean $\pm$ SEM ($n=30$). Different letters indicate statistical differences at $P < 0.05$. Scale bar = 1 cm.

Fig. 5. Effect of biotin on cell viability in the primary root. Seedlings of the transgenic line *AtHisH2B::YFP* were grown for 13 days on agar-solidified $0.2 \times$ MS medium and stained with IP, and cell viability at the apex of the root was analyzed by confocal microscopy. A) Representative micrographs of cells expressing the *AtHisH2B::YFP* gene construct. B) Number of cells expressing *AtHisH2B::YFP*. Data represent the mean $\pm$ SEM ($n=30$). Different letters indicate statistical differences at $P < 0.05$. Scale bar = 50 μ m.

1C). Representative photographs of seedlings visibly showed the reconfiguration of the root system architecture in response to biotin (Fig. 1 d-g).

Biotin alter elongation and cell differentiation

Because biotin modified the growth and development of the radicular system, we analyzed the effect of the treatment on elongation and cell differentiation using seedlings expressing *AtExp7::uidA* (a marker of elongation and differentiation). At 13 days after germination, the seedlings were stained and treated to determine the activity of the reporter gene *uidA* (GUS) directed by the promoter *Exp7*. The meristem length was determined as the distance from the quiescent center to the cell file where cells started to elongate. We observed that as the biotin concentration increased (250 μ M-1000 μ M), the gen GUS was expressed closer to the tip of the root (Fig. 2B). This result indicated that the cells started to

expand into a zone closer to the tip of the root and then lost the capacity to divide; this effect was biotin concentration dependent. The addition of a higher concentration of biotin reduced GUS expression demonstrating that elongation of the primary root was deficient at these concentrations (Fig. 2A).

Biotin regulates the mitotic activity of the primary root

To determine how biotin modifies primary root elongation, we used plants of the transgenic line *CycB1;1:uidA*, with expression during the G2/M phase transition of the cell cycle. Activity of the reporter gene GUS was observed in the meristem region of the principal root (Fig. 3A), and biotin at 1000 μ M decreased the number of expressing cells in the meristem (Fig. 3B).

Fig. 6. Effect of biotin on the formation of reactive oxygen species. Fluorescence (green color) determined at 13 days revealed the presence of ROS in primary root cells. Fluorescence in primary root tips was quantified using the ImageJ program, and the graph is shown in arbitrary units (AU). The roots of 30 seedlings were analyzed by confocal microscopy. Data represent the mean $\pm$ SEM. Different letters indicate statistical differences at $P < 0.05$.

Total fresh weight of foliage

To evaluate the influence of the inhibition of growth in the primary root over aerial development at 13 days after germination, the total fresh weight of the foliage was quantified. Analysis of the total fresh weight of foliage in seedlings treated with biotin (500–1000 µM) resulted in a slight decrease in biomass accumulation compared with the control (Fig. 4A and 4B).

Effect of biotin on cell viability

Due to the inhibition of growth in the primary root, seedlings expressing *AtHisH2B::YFP* histone, which functions as a marker of viability, were assessed. Interestingly, biotin decreased the number of cells expressing *AtHisH2B::YFP* in the meristem. This result supports the findings obtained for *CycB1;1:uidA*. Nevertheless, at higher concentrations

(500–1000 µM), visible cell reduction was detected in the meristematic region, further modifying the morphology (Fig. 5).

Generation of ROS

Reactive oxygen species (ROS) function as signals during the development of plants and perform an important role in the regulation of root growth (Schipper et al. 2012); however, excess ROS exert a phytotoxic effect (Shahid et al. 2014). ROS formation in the tip of the primary root were analyzed using the fluorochrome EROS H2DCF-DA and confocal microscopy. The ROS distribution in the root apex with local maxima in the quiescent center and elongation zone was clearly visible and exhibited biotin concentration-dependent profile (Fig. 6).

Fig. 7. Effect of biotin on leaf total chlorophyll content of ecotype Col-0. *Arabidopsis thaliana* seedlings were grown for 13 days on agar-solidified 0.2 × MS medium, and total leaf chlorophyll content was quantified in the presence of different concentrations of biotin. Data represent the mean ± SEM (n=30). Different letters indicate statistical differences at $P < 0.05$.

Leaf chlorophyll content

Total chlorophyll content in the leaf was quantified to determine whether there was a relationship between the effect of biotin on root viability. In contrast to the results obtained for root viability, the chlorophyll concentration in foliage did not show a significant change at a high biotin dose of 1000 μM (Fig. 7).

Discussion

Several works have studied the additional biological roles of biotin in bacteria, mammals and plants, namely, in regulating gene expression (Cronan 1989, Rodriguez and Zemleni, 2003, Zemleni 2005, Li et al. 2012, Maruyama et al. 2012). The studies reported herein indicate that as observed for another coenzyme, folic acid (Ayala et al. 2017), biotin supplementation has additional biological roles in the modification of root development through regulating gene expression. It is very important to mention that the biotin concentrations used in this study (250 to 1000 μM) are in the range of those used for rescued auxotrophic mutants phenotype and in other experiments (Chen et al. 2003, Muralla et al. 2008, Li et al. 2012, Pommerrenig et al. 2013). Until, biotin deficiency has only been studied in the development of *Arabidopsis thaliana* (Schneider et al. 1989, Patton et al. 1996, 1998, Pinon et al. 2005, Muralla et al. 2008) and the phenotypes are not indirect consequences of biotin-dependent enzymatic reactions that are occurring too slowly or not at all.

Therefore, in the present work, we extended our analysis and show for the first time that biotin supplementation are results in root architecture reconfiguration in *A. thaliana*, acting as a bioactive molecule implicated in signaling mechanisms that modify cell division and development processes within the root.

In mammals, the most widely accepted mechanism responsible for the effects of biotin on gene expression involves a soluble guanylate cyclase-signaling cascade and histone biotinylation (Riveron and Fernandez 2017). It has been previously reported that the expression of certain biotin-containing protein genes is regulated by biotin itself in plants (Chen et al. 2003). However, until now, no specific mechanism for the regulation of gene expression by biotin has been described in plants. Li et al. (2012) proposed three possibilities: 1) histone biotinylation resulting in chromatin remodeling and epigenetic regulation; 2) transcriptome reprogramming producing pleiotropic effects; 3) biotinylated proteins in lipid biosynthesis that produce lipids, which may impact oxidative stress signaling. In this sense, we have previously reported a decrease in the total mass of fatty acid synthase (FAS) enzyme and the phosphorylation of ACC 1 carboxylase. Furthermore, we observed phosphorylation (activation) of AMPK kinase, a key in the control of lipid synthesis (Aguilera and Fernandez 2012). Therefore, mechanisms responsible for the change in root architecture require further study.

Growth of the primary root of seedlings supplemented with higher concentration of biotin

(1000 μ M) was reduced. At 13 days after germination, the length gain was less than 2 cm, while the primary root of wild type seedlings grew more than double this length (Fig. 1A). The determined growth of the primary root of plants treated with biotin was caused by consumption of the meristem (Fig. 1D) in a concentration-dependent manner. This phenomenon can be detected morphologically by the presence of root hairs at the tip of the root formed from differentiated epidermal cells (López et al. 2005). Therefore, biotin can affect different cellular and genetic processes in cell specification, initiation, and elongation of root hair development in *Arabidopsis thaliana*. Similarly, the number of lateral roots increased in a manner that was directly related to the biotin concentration in the medium (Fig. 1B and C), an effect that has been widely reported in cases of depletion of the apical meristem of the root (Sánchez et al. 2006, Shishkova et al. 2008, Gutiérrez et al. 2018). Depletion of the meristem of the root was dependent on the biotin concentration (Fig. 2A and B), and as expected, there was a decrease in the number of dividing meristematic cells during the course of root apical meristem depletion (Fig. 3B). Although the cells of the root meristem still showed mitotic activity at the higher concentration of biotin (1000 μ M) (Fig. 3A), it was lower than in the control group. It is important to remark that an excess of biotin (1000 μ M) triggered differentiation in the meristem, via an alternate cellular program that concludes with cell division in the meristem, inducing cell differentiation. This program is also named “determinate” because when activated root growth can no longer be sustained (Ayala et al. 2017).

Although the excess of biotin slightly decreased amount of foliage (Fig. 4B), this phenomenon cannot be directly related to biotin since these conditions affected the radicular system, in turn leading to slower growth. However, the concentration of chlorophyll in the seedlings did not change with respect to the control and increased in response to a biotin concentration of 1000 μ M (Fig. 7). The production of chlorophyll was not affected, indicating that the slower growth was not a toxic effect of biotin and that the vitamin probably even induces biosynthesis activity of chlorophyll or potentially retards its degradation at the higher concentration (1000 μ M).

The mutant *bio-4* of *Arabidopsis thaliana* (*Atbio F*) has impaired activity of the enzyme 7-ceto-4 amino pelargonic acid synthase, the first enzyme in biotin biosynthesis (Pinon et al. 2005). Although this mutant shows a reduced growth rate and lesions in leaves that are similar to those produced by pathogens (chlorotic and necrotic leaves), the

published literature has reported no growth of the root (Li et al. 2012). Thus, our results differ from these publications. Our results are another example that the developmental program required to maintain the length of the meristem differs from the program required to keep it indeterminate. When the first is comprised, the meristem remains short, but when the second is impaired, the meristem is completely consumed. Therefore, the developmental mechanism of undetermined growth toward determined growth is a developmental pathway that is specific for the regulation of the root apical meristem. We questioned whether biotin could cause determined growth or whether it was simply a secondary effect of the excess vitamin. Viability assays with *AtHisH2B:YFP* showed that the meristem was depleted (Fig. 5). These findings indicated that primary root growth inhibition was likely due to altered cell division and inhibition of cell elongation. Therefore, repression of root development may have originated for an alternate cellular program that terminates cell division in meristems, giving rise to cell differentiation, as previously reported by Ayala et al. (2017) for folic acid supplementation in *Arabidopsis thaliana*.

Since biotin is a cofactor of carboxylases, such as acetyl CoA carboxylase, propionyl CoA carboxylase and pyruvate carboxylase (Smith et al. 2007), an increment in the biotin concentration may raise mitochondrial activity by increasing carboxylase activity. In plants, mitochondria produce ROS (Noctor et al. 2018), which can act like signaling molecules and modulate gene expression. For example, polyunsaturated fatty acids are a preferred target of ROS attack, producing several lipid oxidation products that are bioactive molecules and that can modify gene expression (Pitzschke et al. 2006). In this way, the rise in ROS concentration triggered by biotin (Fig. 6) would lead to the development of root hairs near the root tip and change the morphology of the cells (Fig. 5). The root hairs in the growing root tip and pollen tubes generate ROS, and it has been demonstrated that these molecules are necessary for the growth process (Foreman et al. 2003, Potocký et al. 2007). Moreover, it has determined that the production and accumulation of ROS regulate calcium homeostasis by modulating of Ca^{2+} permeable channels. For example, the tip-focused free cytosolic Ca^{2+} gradient plays a central role in regulating tip growth by facilitating vesicle exocytosis of root epidermal cells (Cárdenas 2009). We recently, reported that biotin in rat aortic rings can modify the extracellular influx and intracellular release of calcium (Aguilera et al. 2019). Therefore, biotin could also regulate calcium homeostasis in plants and modify the growth of root

hairs. Further experiments are in progress in our laboratory to explore this last possibility. Similarly, it is well documented that ROS cause lipoperoxidation, DNA damage, modification of the protein structure, and carbohydrate oxidation (Swanson and Gilroy 2010, Torres 2010, Shahid et al. 2014), further suggesting that ROS overproduction may be responsible for the slow growth of the root in response to biotin.

However, some researchers have recently proposed that the exposure of *Arabidopsis thaliana* roots to light induces stress responses with increased growth rates, so called “escape tropism” strategies (Yokawa et al. 2011, Xu et al. 2013, Yokawa et al. 2014, Zheng et al. 2019). This light-induced root growth is driven by photoreceptors that are active in roots (Yokawa et al. 2013). ROS signaling seem to be involved and acts like an emergency alert, producing signaling events that lead to physiological responses in *Arabidopsis thaliana* roots and can be transferred throughout the plant body (Xu et al. 2013, Yokawa et al. 2013, Zheng et al. 2019). However, we observed a decrease in primary root growth and an increase in lateral roots when roots were incubated with biotin under the same conditions of light exposure with respect to those that were not. We consider these findings to be very important as plant roots are extremely sensitive to light. We are currently standardizing the methodology to test the effect of biotin on roots in the dark.

Conclusion

In conclusion, our data show, for the first time, that biotin supplementation triggers molecular mechanisms that modify cell division and cell differentiation processes within the root and have a profound impact on the root system architecture in *Arabidopsis thaliana*. Biotin supplementation inhibits primary root growth and promotes lateral root formation. Primary root growth arrest by biotin presumably occurs owing to the inhibition of cell elongation and altered cell division. We hypothesize that biotin has additional biological roles, namely, the regulation of gene expression and signaling events. Although the mechanisms by which biotin affects gene expression remain vague, chromatin remodeling by histone biotinylation, reprogramming of the transcriptome and some bioactive molecules produced by lipid oxidation are possible mechanisms that may affect gene expression (Chen et al. 2003). Additionally, localized production of reactive oxygen species (ROS) and calcium homeostasis could play roles in the non-enzymatic functions of biotin. However, we cannot rule out the possibility that other important root hormones that affect root

architecture reprogramming participate in the response to biotin. Therefore, future research is required to determine the effects of biotin on the root architecture changes and other aspects of plant development observed in this study.

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Conflict of Interest Statement

The authors declare that there are no conflicts of interest.

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