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ECO LETTER

BIORADICANTE NOT ONLY INCREASES
ROOT GERMINATION AND ROOT LENGTH

#21

Periodic publication on efficacy and characteristics of Futureco Bioscience products.

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BIORADICANT NOT ONLY INCREASES ROOT GERMINATION AND ROOT LENGTH

INTRODUCTION

The root performs functions of capital importance in crops, such as nutrient uptake and the establishment and anchoring of the plant to the substrate. The main roots have a structural and conduction function of nutrients and water, the absorption of which occurs in the secondary roots and root hairs. The development of an adequate root system determines the survival of plants at transplanting and the utilization of valuable substrate resources, such as water or mineral salts.

A well-developed root is also decisive in overcoming water deficit events as it can increase water uptake by increasing the volume and depth of the substrate explored. In addition, optimizing the growth and development of the root system has a positive impact on the aerial part, since the root system acts as a source of nutrients and a sensor of nutritional availability for the rest of the plant. The extension and architecture of the root system depends largely on the species - in crops it is usually inherited from wild varieties - but exogenous factors such as nutrient availability, substrate type or temperature also modulate root development and architecture.

Bioradicante® is a biostimulant designed and formulated by Futureco Bioscience to optimize root development, with the benefits that this entails for the survival

and use of the substrate resources, not only of the root itself, but also of the rest of the plant tissues.

In order to explore the mechanisms of action of Bioradicante®, the impact of the treatment of broccoli seedlings (*Brassica oleracea* var. *Italica*) with Bioradicante® has been studied both at the phenotypic level and in the transcriptomic profiles of the seedlings.

MATERIALS AND METHODS

Disinfected broccoli seeds (Gentleman F1) were sown in trays honeycombed with peat. The trays were dipped in a 0.05 or 0.1 % Bioradicante® solution four times within one week intervals, starting at the time of sowing. The control was treated the same, but with tap water. Plants were allowed to germinate and grow in the greenhouse with minimum temperatures of 25/15 °C (day/night), relative humidity of 45-65 % and maximum daily photosynthetically active radiation of approximately 100 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

Ten days after the last application of Bioradicante®, samples were taken to visualize the roots and study the transcriptomic profiles of the seedlings. Transcriptomic profiles of Bioradicante®

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0.1 % treated and control plants were determined using Illumina RNA-seq technology, and analysis and visualization of gene expression data were carried out on Sequentia Biotech's A.I.R. platform.

RESULTS AND DISCUSSION

Seed treatment with Bioradicante® improved germination percentage, especially at the highest treatment dose (0.1%).

TREATMENT	DOSAGE	GERMINATION (%)
Control	0%	79%
Bioradicante	0,05%	88%
	0,1%	94%

Table 1. Effect of Bioradicante® on the germination percentage of broccoli seeds.

Bioradicante® treatment also increased seedling root length, especially in plants treated with the highest dose of Bioradicante® (Fig. 1).

In the analysis of transcriptomic profiles, 4210 differentially overexpressed and 4992 repressed genes were identified in Bioradicante®-treated plants compared to control plants. Among these genes, 17 genes coding for high-affinity nitrate transporter enzymes (HATS) were statistically overrepresented (Figure 2, red circles and Table 2), showing an increase in nitrate uptake and transport in broccoli plants treated with Bioradicante®.

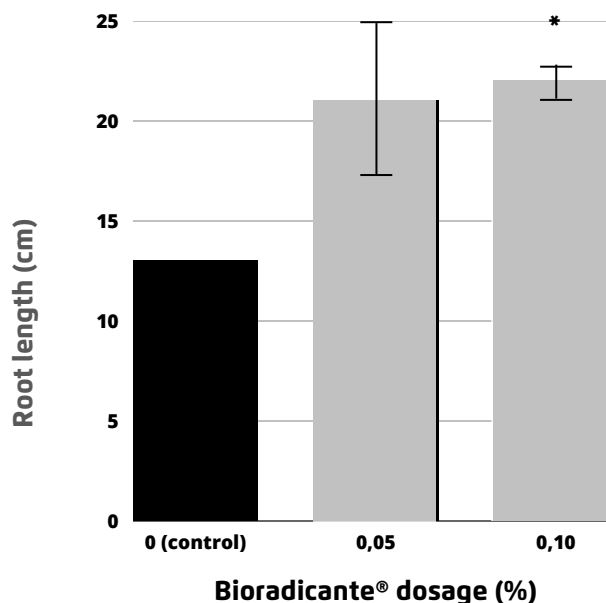


Figure 1. Effect of Bioradicante® on root length of broccoli seedlings. Data are shown as mean \pm standard error of 5 plants. The asterisk indicates statistically significant differences with respect to the control (T-test, $P < 0.05$).

HATS (and in particular the four of them overexpressed: NRT2.1, NRT2.2, NRT2.4, and NRT2.5) function in roots (Figure 3) at very low concentrations (saturating at 0.2-0.5 mM) of external nitrate. Once assimilated in the roots, nitrate can be translocated to aerial organs via the xylem.

On the other hand, the list of overexpressed genes in plants treated with Bioradicante® is strongly enriched in genes with functions located in the cell periphery and associated with cell membrane and cell wall biosynthesis, probably indicating biomass growth (Figure 2, green box).

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Figure 2. Manhattan-plot for broccoli (*Brassica oleracea* var. *italica*) samples treated with Bioradicante®. Each circle shows an enriched gene ontology versus the control, organized into molecular functions (MF, left in red) and cellular compartment (CC, right in green). The transmembrane activity term for N is highlighted with its statistical significance.

Gene.stable.ID	Description
Bo01028s040	PREDICTED: Brassica napus high-affinity nitrate transporter 2.1 (LOC106412400), mRNA
Bo20680s010	PREDICTED: Brassica oleracea var. oleracea high-affinity nitrate transporter 2.1-like (LOC106310928), mRNA
Bo23656s010	PREDICTED: Brassica oleracea var. oleracea high-affinity nitrate transporter 2.1-like (LOC106310928), mRNA
Bo28944s010	PREDICTED: Brassica oleracea var. oleracea high-affinity nitrate transporter 2.1 (LOC106343181), mRNA
Bo29559s010	PREDICTED: Brassica oleracea var. oleracea high-affinity nitrate transporter 2.1-like (LOC106310928), mRNA
Bo2g050860	PREDICTED: Brassica oleracea var. oleracea high-affinity nitrate transporter 3.1-like (LOC106325435), mRNA
Bo2g167790	PREDICTED: Brassica oleracea var. oleracea uncharacterized LOC106324591 (LOC106324591), mRNA
Bo3g042740	PREDICTED: Brassica oleracea var. oleracea high-affinity nitrate transporter 3.1 (LOC106332148), mRNA
Bo5g008770	PREDICTED: Brassica oleracea var. oleracea high-affinity nitrate transporter 2.1 (LOC106343180), mRNA
Bo5g008780	PREDICTED: Brassica oleracea var. oleracea high-affinity nitrate transporter 2.1 (LOC106343181), mRNA
Bo8g010950	PREDICTED: Brassica napus high-affinity nitrate transporter 2.1-like (LOC106433209), mRNA
Bo8g065100	nitrate transporter2.5 [Source:TAIR%3BAcc:AT1G12940](projected from arabidopsis_thaliana%2CAT1G12940)
Bo8g112680	High-affinity nitrate transporter 2.2 [Source:Projected from Arabidopsis thaliana (AT1G08100) UniProtKB/Swiss-Prot%3BAcc:Q9LMZ9]
Bo8g112690	nitrate transporter 2:1 [Source:TAIR%3BAcc:AT1G08090] (projected from arabidopsis_thaliana%2CAT1G08090)
Bo9g096970	PREDICTED: Brassica oleracea var. oleracea high-affinity nitrate transporter 3.1-like (LOC106316673), mRNA
Bo9g146990	nitrate transporter 2.4 [Source:TAIR%3BAcc:AT5G60770] (projected from arabidopsis_thaliana%2CAT5G60770)
Bo9g147000	High affinity nitrate transporter 2.4 [Source:Projected from Arabidopsis thaliana (AT5G60770) UniProtKB/Swiss-Prot%3BAcc:Q9FJH8]

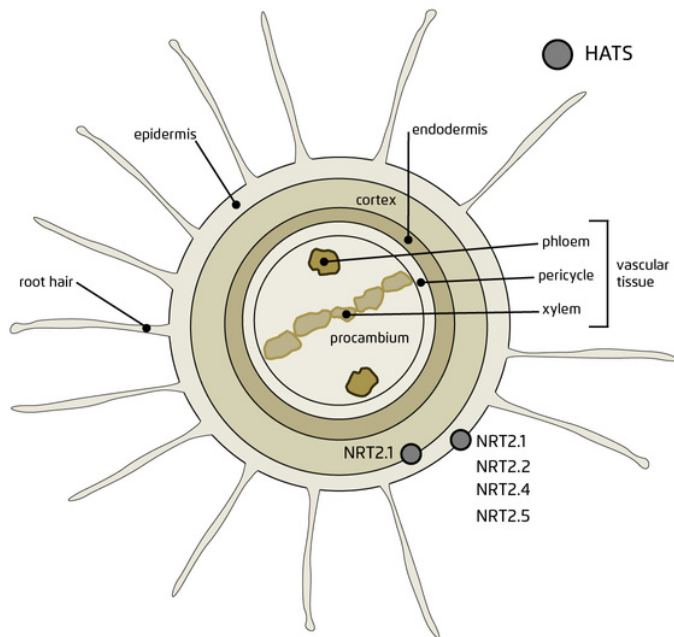


FIGURE 3. Schematic representation of high-affinity nitrate transporters expressed in an Arabidopsis root cutting.

Table 2. List of the 17 overexpressed genes contained in the gene ontology of nitrate transmembrane transporters enriched in Bioradicante®-treated plants.

CONCLUSIONS

Bioradicante® enhances germination and root development in broccoli seedlings.

The effect of Bioradicante® on broccoli seedlings is due, at least in part, to its stimulation of nitrate uptake.

In addition, Bioradicante® modulates cell membrane and cell wall dynamics, a key process for the growth and development of the root system and subsequent aerial growth.



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