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Changes in growth and concentration of amino acids in Chinese cabbage seedlings using bacterial fermented broth

Cambios en el crecimiento y concentración de aminoácidos en las plántulas de col china usando caldo bacteriano fermentado

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ABSTRACT

The biofertilizer derived from the fermentation of sugarcane molasses by the bacterium *Corynebacterium glutamicum* is a broth containing L-glutamic amino acid. To evaluate the biofertilization effect of this broth on the organic production of Chinese cabbage seedlings (*Brassica campestris* var. Pekinensis), an experiment was performed in a seedling nursery in the organic area at the Federal University of Paraná (UFPR). Aqueous solutions of the fermented broth were applied to leaves at concentrations of 0.2 mL L^{-1} and 0.8 mL L^{-1} in addition to the control with water application.

The results indicated that foliar application promoted the development of the aerial part and roots and increased the concentration of total free amino acids in Chinese cabbage seedlings. These results demonstrated the biofertilizing action of the bacterial fermented broth, promoting seedling growth and stimulating amino acid synthesis, by increasing the concentration of total free amino acids.

Key words: *Brassica campestris* var. pekinensis. Biofertilizer. Biostimulant. Organic production.

RESUMEN

El biofertilizante de la caña de azúcar derivado de la fermentación por la bacteria Corynebacterium glutamicum *es un caldo que contiene aminoácido L-glutámico. Para evaluar el efecto biofertilización de este caldo en la producción orgánica de las plántulas de col china (*Brassica campestris *var. Pekinensis), se realizó un experimento en un vivero de plántulas en la zona orgánica de la Universidad Federal de Paraná (UFPR). Las soluciones acuosas del caldo fermentado se aplicaron a hojas a concentraciones de 0,2 mL* L^{-1} *y 0,8 mL* L^{-1} *además del control con aplicación de agua.*

Los resultados indicaron que la aplicación foliar promovió el desarrollo de la parte aérea y las raíces y aumentó la concentración de aminoácidos libres totales en las plántulas de col china. Estos resultados demuestran la acción biofertilizante del caldo bacteriano fermentado, promoviendo el crecimiento de las plántulas y estimulando la síntesis de aminoácidos, aumentando la concentración de aminoácidos libres totales.

***Palabras clave**:* Brassica campestris *var. pekinensis. Biofertilizante. Biostimulante. Producción orgánica.*

Introduction

The family Brassicaceae has the greatest diversity among the vegetable species grown in Brazil (Filgueira, 2003), which includes Chinese cabbage (*Brassica campestris* var. *Pekinensis*). This species stands out for its high nutritional value, being an excellent source of folic acid, vitamin A, vitamins B and C, as well as calcium and potassium (Embrapa, 2010).

The production of seedlings is an important step in the cultivation of vegetables, a phase in which the development and adequate growth of plants contribute to the success and establishment of the crop (Alvarenga, 2013; Borcioni *et al*., 2016). In addition, it contributes to the reduction of the cycle in the field, since the seedlings have the root system and aerial part growing compared to direct sowing in beds (Guerra *et al*., 2016).

Studies focusing on the use of promoting effect products derived from fermentative processes on plant growth are rare; however, recent studies have shown that bacterial fermentation of cane

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molasses containing the amino acid L-glutamic acid promoted increases in the biomass of the aerial part and the growth of the roots of the seedlings, characterizing it as a biofertilizer (Bettoni *et al.*, 2014; Röder *et al.*, 2015).

Biofertilizers are defined in Normative Ruling N° 46 (Mapa, 2011) as products that contain active components or biological agents capable of acting, directly or indirectly, on the complete or on parts of the cultivated plants, improving the performance of the production system The use of biofertilizers in the production of seedlings may be considered as a sustainable alternative, especially in an organic system. The objective of this study was to determine the biometric changes in Chinese cabbage seedlings submitted to foliar applications of a broth containing the amino acid L-glutamic acid resulting from the fermentation of sugarcane molasses by the bacterium *Corynebacterium glutamicum* (Dreyer *et al.*, 2000). In addition, the total free amino acid and chlorophyll content in the leaves of the seedlings were determined as possible biochemical indicators of the biofertilizer action.

Material and Methods

The experiment was carried out in protected cultivation, in a bench traditionally used for the commercial production of seedlings with timed micro-sprinkling irrigation, in the Organic Area of Canguirí Farm - Federal University of Paraná, located in the city of Pinhais, Paraná state (25º 25’ S, 49º 06’ W at an altitude of 920 m). Seeds of “Yuuki” (Takii Seeds), a Chinese cabbage hybrid with 95% germination were used. Sowing was carried out in expanded polystyrene trays with 200 cells, filled with a substrate composed of composted poultry litter (Provaso®), combined with composted pine bark in a ratio of 1:1.5, respectively (Bettoni *et al.*, 2014).

The treatments consisted of foliar applications of aqueous solutions at the concentrations of 0.2 $ml.L^{-1}$ and 0.8 $ml.L^{-1}$ of sugarcane molasses broth fermented by the bacterium *Corynebacterium glutamicum* (AG30), which presents 30% (m\v) of the amino acid L-glutamic acid and 6% organic carbon (Microquímica Indústrias Químicas Ltda.), as well as a control with application of distilled water. The application was made 15 days after sowing, when the first true leaves appeared. A pressurized spray with CO_2 and constant pressure of 45 pounds pol^{-1} was used with a spray volume of 100 mL per treatment (Röder *et al.*, 2015).

The experimental design was completely randomized, composed of three treatments with 10 replicates, using five plants per replicate for the evaluations. At 22 days after sowing (DAS), when the seedlings had produced the fifth true leaf, the plants were collected and the following variables measured: aerial part fresh mass, aerial part dry mass, root fresh mass, aerial part dry mass and root dry mass ratio, root volume, leaf area, total free amino acid content and total chlorophyll in leaf tissue.

The roots of the seedlings were washed to remove the adhered substrate. Then the plants were sectioned, separating the aerial part from the root system for the evaluation of fresh mass. Leaf area and root volume were analyzed using WinRhizo® software coupled to a Scaner LA1600 (Régent Instruments Inc. 2011); the leaves were read at 50 DPI resolution and the roots at 150 DPI (Szilagyi-Zecchin *et al.*, 2015). The dry mass was obtained by drying the plants in an oven at a temperature of 65 ºC ± 5 ºC and forced air circulation until constant weight, and then weighing on an analytical scale.

Total free amino acids were extracted in distilled water (Winters *et al.*, 2002) and quantified by reaction of the sample with 0.2 M citrate buffer pH 4.6 and ninhydrin solution (1% ninhydrin, 0.03% ascorbic acid in 2-methoxyethanol) (Magné and Larher, 1992). The readings were performed at 570 nm (Magné and Larher, 1992) in a spectrophotometer (BEL2000uv®). The standard curve was performed with 4 mM glutamine with values ranging from 28 to 140 µg/mL, yielding the equation: $y = 0.01\ x + 0.0741$, with $R^2 = 0.9908$. Pigments were extracted according to Lichtenthaler (1987) with 80% acetone in distilled water plus 0.1% $CaCO_3$ (w/v) (Pompelli *et al.*, 2013). The readings were carried out at 663 and 647 nm in the same spectrophotometer previously mentioned. The following formulas were applied (Lichtenthaler and Buschmann, 2001):

$$[Cl\ a] = 12.25 \times A663\ nm - 2.79 \times A647\ nm \quad (1)$$

$$[Cl\ b] = 21.50 \times A647\ nm - 5.10 \times A663\ nm \quad (2)$$

$$[Cl\ a + b] = 18.71 \times A647\ nm + 7.15\ A663\ nm \quad (3)$$

Data were submitted to analysis of variance and for the variables that presented significant differences, the Scott-Knott test was performed using a significance level of 0.01. Data were processed by ASSISTAT software, version 7.7 Beta (Silva and Azevedo, 2002).

Results and Discussion

The fresh mass of the aerial part and roots of Chinese cabbage seedlings showed a significant increase on the order of 58% and 42%, respectively, when submitted to the application of the bacterial fermented broth containing 30% L-glutamic acid (AG30) at a concentration of 0.2 mL.L^{-1} (Figure 1). A similar result was obtained by Bettoni *et al.* (2014) with the use of bacterial fermented containing L-glutamic acid, which produced an increase in the aerial part and the root system of *Origanum vulgare* seedlings. Effects of the exogenous application of L-glutamic acid on growth promotion and on biochemical changes related to N metabolism in plants were reported by Yu *et al.* (2010), relating plant growth to the absorption of the amino acid that converted to glutamate provides greater cell division.

Seedlings treated with 0.2 mL.L^{-1} AG30 showed a significant gain of 78% in the dry mass of the aerial part compared to the control (Figure 2).

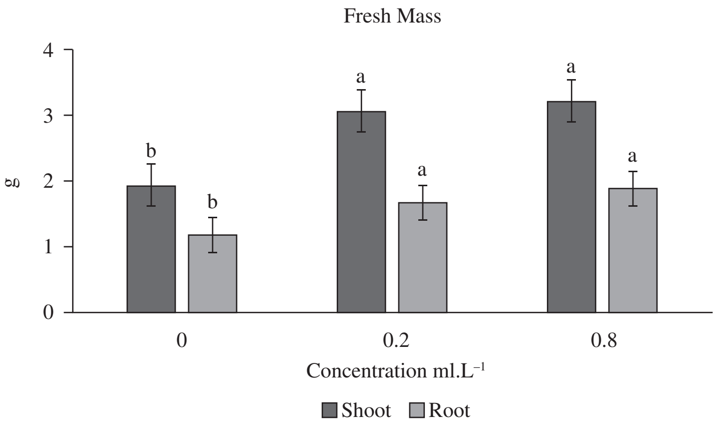

Figure 1. Effect of foliar application of different concentrations of bacterial fermented broth on fresh shoot and root mass of Chinese cabbage seedlings cv. Yuuki. Bars topped by the same letter do not differ significantly by the Scott-Knott test at P < 0.01. (Shoot F = 51.7792 **; Root F = 14.7380**).

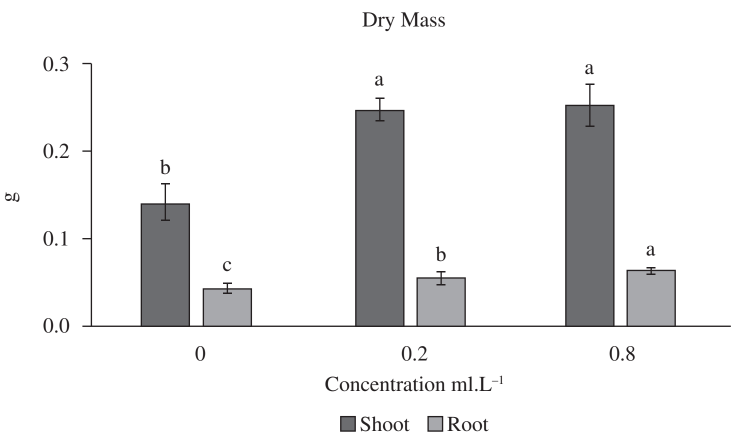

Figure 2. Effect of foliar application of different concentrations of bacterial fermented broth on dry shoot and root mass of Chinese cabbage seedlings cv. Yuuki. Bars topped by the same letter do not differ significantly by the Scott-Knott test at P < 0.01. (Shoot F = 109.1052 **; Root F = 31.9026**).

Similarly, Fabbrin *et al.* (2013) verified an increase in the dry mass of the aerial part of *Cichorium intybus* L seedlings after the application of AG30 at a concentration of 0.8 $mL.L^{-1}$. Cardoso *et al.* (2014) also observed accumulation of 10% of dry matter per hectare after applying biofertilizer containing L-glutamic acid in the cultivation of *Avena sativa*.

The dry mass of the roots in the seedlings submitted to the treatment with 0.8 $mL.L^{-1}$ showed an increase of about 55% compared to the control (Figure 2). Colla *et al.* (2014) treating tomato stakes with a biofertilizer based on amino acids at a concentration of 6 $ml.L^{-1}$, found a greater accumulation of dry mass of the aerial part and roots compared to the control; this result was related to the absorption and metabolization of amino acids present in the biofertilizer.

The biomass partition of plants may be represented by the ratio between the dry mass of the aerial part and the dry mass of the roots. Guzmán-Antonio *et al.* (2012) discussed the behavior of this ratio after fertilizing (N, P, K) habanero pepper seedlings since there is an increase in nitrogen supply; this ratio was greater compared to the treatments with no addition of N. We verified that the seedlings submitted to AG30 at the concentration of 0.2 $mL.L^{-1}$, exhibited a higher index (4.63) compared to the other treatments (Figure 3). This result indicates that the application of the biofertilizer led to greater growth of the aerial part of the seedlings in relation to the roots. Bettoni *et al.* (2013), applied 8 $ml.L^{-1}$ of a protein hydrolyzate containing amino acids to leaves of broccoli, finding greater increases of the fresh and dry mass of the aerial part of the plants. The authors related the result to the action of amino acids in plants because they involved in the synthesis of enzymes and plant hormones.

Application of 0.2 $mL.L^{-1}$ AG30 increased leaf area by 47% compared to the control (Figure 4). Röder *et al.* (2015) found similar results using bacterial fermented containing L-glutamic acid in cabbage seedlings. However, at a higher concentration (0.8 $mL.L^{-1}$), leaf area increased by 22% compared to the control with no application. These results are expected, considering that L-glutamic acid is a precursor of glutamine and glutamate in nitrogen metabolism, and may act to increase the leaf area and aerial part growth (Kerbauy, 2008).

Root volume of the Chinese cabbage seedlings showed a significant increase at the concentration of 0.8 $mL.L^{-1}$ of AG30 compared to the other treatments (Figure 5). This result is similar to that found by Fabbrin *et al.* (2013) in the cultivation of chicory seedlings and by Bettoni *et al.* (2014) in oregano seedlings submitted to the application of bacterial fermented. This effect is desirable, since the increase of root volume in seedlings is directly associated with greater soil exploration and nutrient absorption (Nibau *et al.*, 2008).

Application of AG30 did not change the concentration of chlorophyll A, B or the total

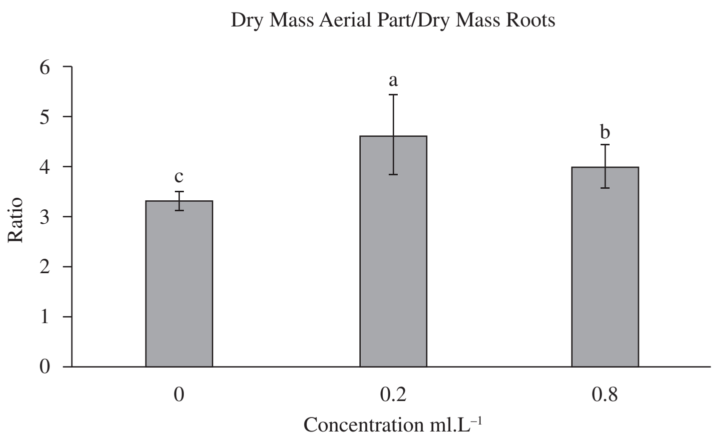

Figure 3. Effect of foliar application of different concentrations of bacterial fermented broth on the ratio of dry mass of shoots to dry roots of Chinese cabbage seedlings cv. Yuuki. Bars topped by the same letter do not differ significantly according to Scott-Knott test at $P < 0.01$. (Ratio $F = 17.0680^{**}$).

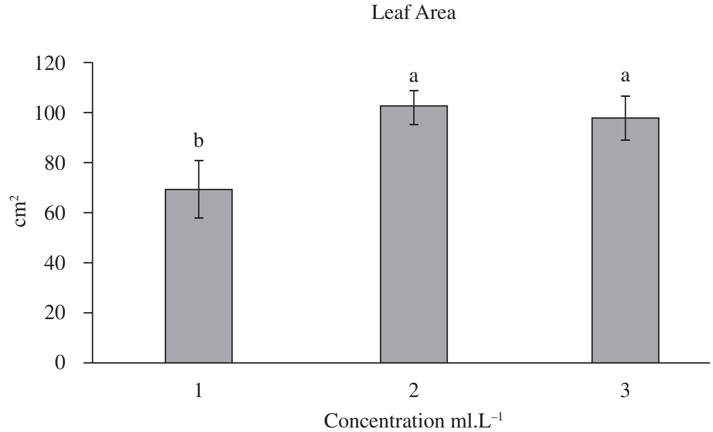

Figure 4. Effect of foliar application of different concentrations of bacterial fermented broth on leaf area of Chinese cabbage seedlings cv. Yuuki. Bars topped by the same letter do not differ significantly according to Scott-Knott test at $P < 0.01$. (Leaf area $F = 37.6442$**).

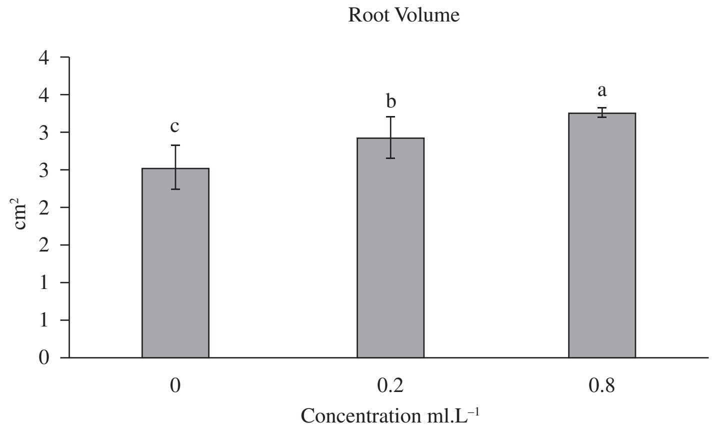

Figure 5. Effect of foliar application of different concentrations of bacterial fermented broth on root volume of Chinese cabbage seedlings cv. Yuuki. Bars topped by the same letter do not differ significantly according to Scott-Knott test at $P < 0.01$. (Root Volume $F = 25.8063$**)

in the leaf tissue of Chinese cabbage seedlings. The averages of chlorophyll A, chlorophyll B and total chlorophyll of treatments were 2.09; 1.42 and 3.51 $mg.g^{-1}$ for the control, 1.79; 1.22 and 3.01 $mg.g^{-1}$ for the treatment with 0.2 $mL.L^{-1}$ and 1.83, 1.26 and 3.09 $mg.g^{-1}$ for the treatment with 0.8 $mL.L^{-1}$ of the bacterial fermented. Although glutamic acid is a precursor to chlorophyll synthesis in developing leaves (Yaronskaya *et al.*, 2006), no effect of the amino acid on altering chlorophyll concentration has been found. However, a significant gain was observed in the relative chlorophyll index compared to the control in seedlings of *Brassica oleracea* var. capitata submitted to the application of bacterial fermented containing L-glutamic acid (Röder *et al.*, 2015).

The application of AG30 promoted a significant increase in the concentration of free amino acids in the leaves of Chinese cabbage seedlings. In the treatment with the concentration of 0.2 $mL.L^{-1}$ AG30, a fivefold increase in total free amino acids of the leaves was found compared to the control (Figure 6). Ertani *et al.* (2013) reported that the application of biofertilizer containing amino acids in the cultivation of corn at a concentration of 0.1 $mL.L^{-1}$ increased the amino acid content in the leaves and roots in relation to the control, indicating the increase in amino acid synthesis

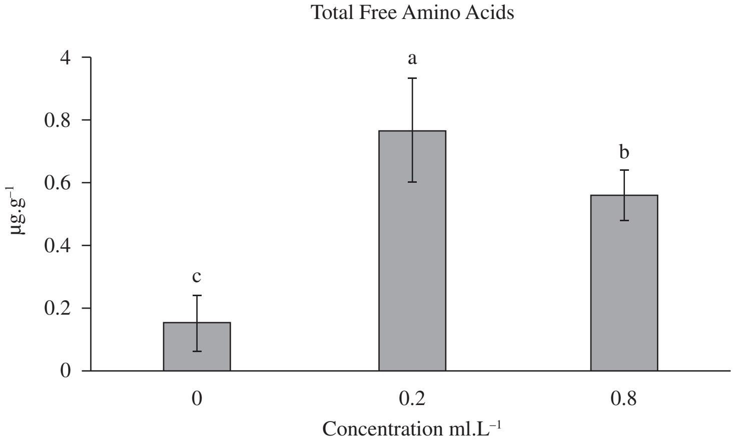

Figure 6. Effect of foliar application of different concentrations of bacterial fermented broth on total free amino acids in Chinese cabbage seedlings cv. Yuuki. Bars topped by the same letter do not differ significantly according to Scott-Knott test at $P < 0.01$. (Amino $F = 35.5544^{**}$).

due to the low concentration applied. According to Brandão (2007), increases in productivity obtained by the use of amino acids via leaves in crops are justified because once absorbed and metabolized, amino acids may participate in the synthesis of several compounds with direct effect on plant growth, justifying the results of growth promotion obtained in this study.

Our results show that AG30 foliar application was efficient in promoting development of the aerial part, roots and the concentration of total free amino acids in leaf tissue of Chinese cabbage seedlings conducted in an organic system, related to the increase in free amino acid content.

Conclusions

The use of the biofertilizer derived from bacterial fermentation containing 30% of the amino acid L-glutamic acid promoted significant growth of the aerial part, roots and total free amino acid concentration of Chinese cabbage seedlings. The treatment at the concentration of 0.8 $mL.L^{-1}$ presented greater increases in the root system without a reduction of the aerial part, which is a desirable effect. The free amino acids in the leaves may be biochemical indicators of the biofertilizer action as a function of their increase in the leaves treated with the fermented broth.

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