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Investigation note

Effect of plant growth-promoting rhizobacteria on agronomic and macronutrient parameters in pak choi

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Abstract

Pak choi is a vegetable of high nutritional value and palatability, which has promoted the increase of its cultivation and consumption outside Asia, its region of origin. This work evaluated the effect of the inoculation of three strains of plant growth-promoting rhizobacteria (PGPR) *Arthrobacter pokkalii* JLB4, *Pseudomonas tolaasii* P61 and *Pseudomonas tolaasii* A46 on pak choi plants under controlled conditions in a high tunnel in 2021. Pak choi seedlings without inoculation were used as a control and two inoculations were performed, the first immediately after transplantation of the seedlings into bags with 5 kg of soil and the second one month later. The plants were simultaneously fertilized with inoculation with NPK in a ratio 164-53-187. The results obtained showed that the strain *Pseudomonas tolaasii* P61 was the one that had the greatest effect on dry weight (23.04 ±0.057 g plant^{-1}), as well as on the content of P (0.1704 ±0.001 g plant^{-1}) and S (0.1847 ±0.004 g plant^{-1}) compared to the control, with increases of 11.5%, 10.8% and 12.3%, respectively. The strain *Arthrobacter pokkalii* JLB4 caused greater root growth, the root volume (75 ±5 ml) was 49% higher than the control. However, it had no effect on the dry weight of the aerial part. The results suggest that the combined use of fertilizer and PGPR leads to greater accumulation of biomass and nutrients, such as P, K and S, than the application of fertilizer alone.

Keywords: *Arthrobacter pokkalii*, *Brassica rapa* subsp. *chinensis*, *Pseudomonas tolaasii*.

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Pak choi (*Brassica rapa* subsp. *chinensis*) is an herbaceous plant that belongs to the genus *Brassica* and shares species with other plants or cultivars found in Mexico. Pak choi is grown mainly in Asia and Oceania, where it is considered one of the most important vegetables (Qing *et al*., 2000). Brassicaceae are a group of plants that includes cabbages, kale, broccoli, turnip and cauliflower. Pak choi is widely classified with other Brassicaceae in FAOSTAT under code 0358. There is little information about the cultivation of this plant outside the countries where it is mostly produced. However, its distribution in other countries and continents is mainly due to Asian immigrants, in addition, the consumption of pak choi has increased with the current trend of consuming foods with high nutritional value.

In Mexico, it is produced to meet local demand and export to the United States of America, Canada and Japan. This is carried out in Baja California, Sonora, Aguascalientes and Guanajuato, with the latter state being the one that reports official figures. In 2021, there was a reported production of 20.73 t in the state of Guanajuato, with a yield of 24.56 t ha^{-1} at a price of $5 850.00 MXN per tonne, according to information from the Agrifood and Fisheries Information Service (SIAP, for its acronym in Spanish). The pak choi crop requires well-draining loamy soil, with a pH of 5.8 to 7.5. Nutritional requirements are 55-75 kg ha^{-1} N, 40-80 kg ha^{-1} P and 80-110 kg ha^{-1} K when planting, irrigation 3 times a week and a population of 50 000 plants ha^{-1} (Maseko *et al*., 2017).

About 80% of the bacteria present in the rhizosphere are capable of producing IAA (Vega-Celedón, 2016), these microorganisms interact with plants through the roots and can regulate their development through an imbalance of auxins, affecting the plant positively or negatively. On the other hand, soil bacteria are also able to solubilize phosphates, form chelated compounds, fix atmospheric nitrogen, which means a benefit for plants. These microorganisms are known as plant growth-promoting rhizobacteria (PGPR).

The use of PGPR is an alternative to promote plant growth through the production of growth regulators, solubilization of soil nutrients, tolerance to biotic and abiotic stress, biopesticide and stimulation of plant defenses (Tsukanova *et al*., 2017; Leontidou *et al*., 2020). In this way, the yield of arable areas can be improved by making the use of fertilizers more efficient, this due to the high cost they represent today. In addition, soil damage from chemical fertilizers can be mitigated (Basu *et al*., 2021). The objective of this work was to evaluate the effect of inoculation of three strains of PGPR on the parameters of growth and content of N, P, K and S of pak choi plants in pots and high tunnel conditions.

The experiment was established at the Montecillo campus of the Colegio de Postgraduados (19° 27’ 38.975 north latitude, 98° 54’ 14.84 west longitude and 2 250 m) in February 2021, with an average temperature of 24 °C and relative humidity of 56%. Seeds of pak choi (*Brassica rapa* subspecies *chinensis* L.) variety Cantón were sown, they have a declared germination percentage of 96% (Harley Seeds, United Kingdom) and an experimental germination percentage of 94%. These were placed in a seedbed of 200 cavities that were previously filled with a mixture of perlite and peatmoss (1:1). When the seedlings emerged, they were irrigated with 50% Steiner solution. The 35-day-old seedlings were transplanted into bags containing 5 kg of medium-textured, carbonate-free loamy-clayey-sandy soil, pH of 7.92. The experiment is based on a completely randomized design with 10 repetitions for each treatment and for the control.

Three strains of plant growth-promoting rhizobacteria from the Soil Microbiology Laboratory of the Colegio de Postgraduados were used: *Arthrobacter pokkalii* JLB4 (GenBank access code: MW629814.1) isolated from soil of the rhizosphere of *Solanum lycopersicum*, *Pseudomonas tolaasii* P61 (GenBank access code: KY933651.1) and *Pseudomonas tolaasii* A46 (GenBank access code: KY933652.1) isolated from the rhizosphere of *Solanum tuberosum*. The inoculum was prepared by sowing the strains in a nutrient broth, incubating at 28 °C and stirring at 140 rpm for 72 h. The inoculum density was adjusted to 10^8 CFU ml^{-1} for each strain, quantifying the number of cells in the Neubauer chamber.

Ten pak choi plants were inoculated with 5 ml of inoculum, the controls were inoculated only with sterile nutrient broth, this process was repeated after 32 days following the same procedure. The plants were irrigated every third day with water. All plants were fertilized with a dose of 164-53-187 NPK, one immediately upon transplantation and the first inoculation, the next after the second inoculation.

After three months, the plants were harvested to evaluate the effect of the rhizobacteria. The root volume was determined by the method of volume of water displaced in a graduated cylinder. The material was dried in a forced-air oven at 70 °C for 72 h and then the dry weight of the plant's biomass was determined. The contents of nitrogen (N), potassium (K), phosphorus (P) and sulfur (S) in the plant were evaluated, taking samples of 0.5 g of dry and ground material from the aerial part and roots of the plants. For nitrogen digestion, a mixture of H_2SO_4 and $HClO_4$ in a 2:1 ratio was used, plus H_2O_2 at 30%. For the digestion of K, P and S, HNO_3 and $HClO_4$ were used in a 2:1 ratio. Both digestions and determinations were performed according to the method of Alcántar and Sandoval (1999), with an inductively coupled plasma optical emission spectrometry equipment (ICP-OES 725-ES, Agilent, Santa Clara, CA, USA).

The data obtained were analyzed with the statistical program SAS version 9.0, being an analysis of variance with a post-hoc Tukey mean comparison for the parameters of dry leaf weight, total dry weight and content of N, P, K and S. An analysis for non-parametric data was performed (Kruskall-Wallis comparison) for root volume and dry root weight respectively, with a post-hoc Dunn mean comparison test, with a 95% confidence interval.

Figure 1 shows agronomic parameters related to the interaction of plants with PGPR. In section 1a, a comparison of the root volume and dry root weight of the different treatments with the control is made, it shows that the plants inoculated with strain JLB4 (*A. pokkalii*) were the ones that had the highest dry weight, being 8.51 g, a value between 30 and 47% higher than the other treatments. For root volume, it was also higher, being 75 ml with a difference between 35 and 51% compared to the other treatments. In section 1b, the results of the total dry weight in pak choi leaves for each treatment with PGPR and the control, without a statistically significant difference (p= 0.0562).

The increase in root volume in inoculated plants may be related to a direct growth-promoting mechanism, as is the case of the production of indole-3-acetic acid (Grover *et al*., 2020). Auxins are molecules that actively participate in all phenological stages of plants, at the cellular level they are involved in cell elongation, division and differentiation (Ljumg, 2013). Because this hormone is distributed unevenly in plant tissues and this affects morphogenetic processes, it is known as a ‘morphogen’ (Vanneste and Friml, 2009). The ability of bacteria of the genus *Pseudomonas* and *A. pokkalii*, among others, to produce auxins, which influence root development and architecture, has been recognized (Malik and Sindhu, 2011; Krishnan *et al*., 2016).

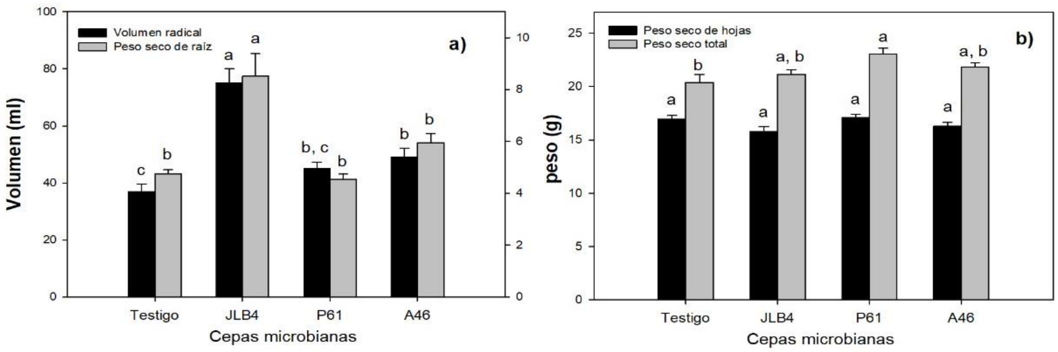

Figure 1. Agronomic parameters in pak choi plants with PGPR treatment. a) root volume and dry root weight; and b) dry leaf weight. Different letters indicate significant differences between groups.

A more abundant root system benefits the anchoring and support of the plant to the soil, a greater contact surface allows the plant to explore the soil, absorb water and nutrients, as well as greater interaction with soil microorganisms. The results of soil-plant-microorganism interactions are complex and will differ depending on factors such as soil type, geographical location, phenological stage and health of the plant (Gomes *et al*., 2018).

The plants inoculated with strain P61 presented the highest total dry weight of all treatments (Figure 1b), with a percentage of 11.5%, unlike the control. Although it produced less root development, in the end it allowed a greater development of the total biomass, which is a desirable effect in vegetables whose aerial part is consumed, as in the case of pak choi. For the elements evaluated in plants, in the case of nitrogen there are no significant differences between the treatments and the control (p= 0.4265) (Figure 2a).

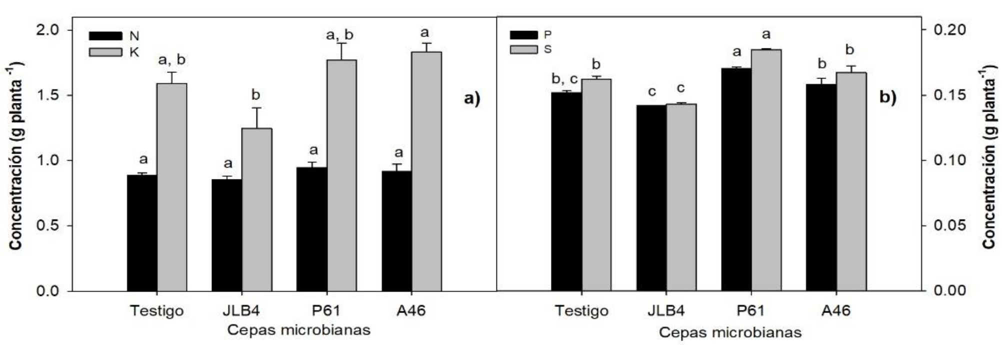

Figure 2. Nutritional content in pak choi plants with PGPR treatment, they are: a) nitrogen (N) and potassium (K), b) phosphorus (P) and sulfur (S). Different letters indicate significant differences between groups.

Nitrogen is an important element in plant development, a deficiency of N can cause proteolysis in leaves, degradation of chloroplasts and decrease in chlorophyll content (Almeida *et al*., 2020). According to the results obtained (Figure 2a), there are no significant differences in the content of this element, which suggests that there is no limitation in the availability of this element. Potassium is a cation with high mobility in plants and with relevant physiological functions, such as the movement of water, nutrients and carbohydrates within the plant (Xu *et al*., 2020). In Figure 2a, it can be seen that, although strain A46 is the one that accumulated the highest amount of K (p= 0.0299), in the post-hoc test no differences were observed between the groups.

A noteworthy characteristic of glucosinolates implies health benefits when intended for human consumption and that they are part of all Brassicaceae (Johnson *et al*., 2016). These metabolites have the main function in the plant as a defense against infections and herbivory (Ishida *et al*., 2014). Zang *et al*. (2015); Blažević *et al*. (2020) mention the synthesis of glucosinolates, which takes place in plants from sulfur-containing amino acids, such as methionine and tyrosine.

In Figure 2b, it can be seen that the treatment with strain P61 produced the highest concentration of this element in plants (p< 0.0001) being 0.1847 ±0.004 g plant^{-1}, 12.3% higher than the control. Although potassium and phosphorus are abundant in the soil, they are bound to other minerals and this causes between 95 and 99% of these elements to be in insoluble forms and not available to plants (Wang *et al*., 2020). Species of the genus *Brassica* can tolerate soils with low availability of phosphorus (P) with fewer adverse effects than other crops with higher requirements (Marschner *et al*., 2007). Nevertheless, PGPR inoculation promotes the solubilization of P and its availability to the plant (Figure 2b).

P. tolaasii P61 produced an increase in phosphorus content, being 0.1704 ±0.001 g plant^{-1}, which was 10.8% higher compared to the control (p= 0.0003). According to Castagno *et al*. (2021), *P. tolaasii* is a bacterium with the ability to promote phosphorus uptake in plants, as is *A. pokkalii* (Alexander *et al*., 2019). This is done by one or both of the following mechanisms to solubilize phosphate: production of low molecular weight acids such as citric acid or gluconic acid in the case of inorganic phosphates or by the synthesis of phosphatases such as phytases or nucleases for organic phosphates (Lobo *et al*., 2019).

Conclusions

In the case of pak choi, the use of the strains *P. tolaasii* P61, *A. pokkalii* JLB4 and *P. tolaasii* A46 in combination with fertilizer led to greater accumulation of biomass, root volume and P, K, S, compared to the application of fertilizer alone. The best strain was *P. tolaasii* P61, which can be used to increase the biomass of pak choi leaves and their nutritional content.

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