

# Effect of the application of plant growth-promoting microorganisms on the cultivation of two varieties of chard (*Beta vulgaris* L. subsp. *cicla*) under greenhouse conditions

## Abstract

Agroecological and sustainable vegetable production arises from the need to produce safe food and protect the environment and with the aim of obtaining sustainable and environmentally friendly agriculture but achieving the best productivity results. Therefore, this work aimed to study the effect of the application of the consortium of native microorganisms on the quality and yield of the crop of two varieties of chard (*Beta vulgaris* L. subsp. *cicla*) at the time of transplantation. The trial was carried out in the greenhouse of the Faculty of Agricultural Sciences of the National University of Catamarca. Swiss chard of the white and rainbow varieties was sown in germination trays. The seedlings were transplanted in beds and inoculated with native plant growth-promoting microorganisms at the time of transplantation. Fresh weight and number of leaves per plant and time of harvest were recorded. The design was in randomized blocks with 4 replications; data were subjected to analysis of variance and LSD test ( $p \leq 0.05$ ). Plants inoculated with the microbial consortium produced significantly higher yields than control plants. The number of cuts and the yield of the Rainbow variety increased significantly progressively with inoculation.

**Keywords:** Swiss chard Penca blanca, Arco iris chard, *Azospirillum brasilense*, mycorrhizal fungi

Volume 11 Issue 2 - 2024

Di-Barbaro MG, Andrada HE, Espeche Acosta ER, Batallán Morales SR, González Basso MV, Godoy Ovejero L, Guzmán PS

Faculty of Agricultural Sciences, National University of Catamarca, Argentina

**Correspondence:** Di-Barbaro MG, Faculty of Agricultural Sciences, National University of Catamarca, Belgrano Avenue and Maestro Quiroga, (4700). Catamarca, Argentina, Email gabydibarbar@yahoo.com.ar

**Received:** March 26, 2024 | **Published:** April 12, 2024

## Introduction

In a world where environmental awareness and the search for more responsible agricultural practices are constantly growing, the use of inoculants from microorganisms selected for their ability to promote plant growth is presented as an innovative and promising solution.

The use of Plant Growth Promoting Microorganisms (MPCV) has been researched for many years, with the genus *Azospirillum* being one of the most prominent, mainly due to its ability to produce a wide range of active metabolites such as phytohormones and other plant growth regulators, such as indole acetic acid, cytokinins, gibberellins and siderophores,<sup>1,2</sup> which positively influence healthy plant growth and development.<sup>3</sup>

Among the MPCVs, there are fungi that establish mycorrhizal associations with the roots of most land plants. These mycorrhizal fungi receive carbon compounds from the plant and in return promote plant growth by supplying nutrients from the soil, especially the few mobile ones such as phosphorus and water.<sup>4-9</sup> In addition, they confer greater tolerance to pathogen attack and drought.<sup>10-11</sup> Mycorrhizae have an advantage over the non-mycorrhizal root because the outer mycelium extends farther than the root hairs, which, from a nutritional point of view, the benefit is the greater growth of the plants due to an increase in phosphorus uptake when this element is limiting, when phosphorus is not limiting the benefit can be null or reduced, according to the degree of mycorrhizal dependence of the plant. In addition, it directly or indirectly influences the absorption of other minerals (N, K, Ca, Mg, Fe, Mn).<sup>3,12</sup>

Swiss chard (*Beta vulgaris* L. subsp. *cicla*) is a biannual vegetable belonging to the Amaranthaceae family, it is a traditional crop that is grown under a conventional system of field production, and highly

appreciated by consumers for its antioxidant and anti-cancer properties. However, it is a crop that has been little researched locally, so it is of interest to check the response of the plant to growing conditions. The aim of this study was to study the effect of the application of the consortium of native plant growth promoting microorganisms (MPCV) on the quality and yield of the crop of two varieties of Swiss chard (*Beta vulgaris* L. subsp. *cicla*) at the time of transplantation.

## Material and methods

The trial was carried out in the greenhouse of the Faculty of Agrarian Sciences of the National University of Catamarca located in the city of San Fernando del Valle de Catamarca (Argentina), in this experiment we worked with two varieties of chard (*Beta vulgaris* L. subsp. *cicla*) Penca Blanca and Arco Iris, a traditional variety (Penca blanca) and a new variety little known in the region (Arco iris). In greenhouse conditions and under a production system with an agroecological approach. It was cultivated together with other companion species, such as aromatics, aphid and ant repellents, pollinators, etc., such as basil (*Ocimum basilicum*), parsley (*Petroselinum crispum*), tufted flower (*Tagetes patula*), garden phlox (*Phlox sp.*). In addition, the conservation and rational use of water resources was promoted, no agrochemicals were used, to achieve a healthy vegetable, a safe food, and the protection of the environment.

Sowing was carried out in germination trays and the seedlings were transplanted into beds with substrate composed of soil, compost, and perlite. Inoculation with native MPCVs was performed at the time of transplantation. Fresh weight and number of leaves per plant and by time of harvest were recorded. The design was in randomized blocks, with 2 treatments, **inoculated:** with application of microbial consortium of MPCV at the time of transplantation, and **control** treatment without inoculation; and with 4 replications.

The microbial consortium was composed of native microorganisms: the endorhizospheric bacterium of the genus *Azospirillum* and mycorrhizal fungi. Inoculation was carried out with the native Pi 8 strain of *A. brasilense*, isolated from the endorhizosphere of pepper (*Capsicum annum* var. Trompa de elefante) grown in the Province of Catamarca, whose identification was carried out biochemically and molecularly.<sup>1,13,14</sup> The concentration of *A. brasilense* used for inoculations was  $2.5 \times 10^7$  azospyrils. mL<sup>-1</sup> quantified in the Neubauer chamber.<sup>15</sup> The inoculum of mycorrhizal fungi native to the province of Catamarca was made up of rootlets of *Avena sativa* L. and *Cenchrus ciliaris* L. colonized by them. The percentage of mycorrhizal colonization of the roots used as inoculum was 85%, estimated using the method of line intersections and microscopic observation of roots by Sieverding, et al.<sup>16,17</sup>

Leaf harvesting was carried out at 60, 65, 70, 80, 90, 100, 120, 130 and 145 days after planting, and based on the total fresh mass per plant, the yield of each treatment was estimated.

Data were subjected to analysis of variance and LSD test ( $p \leq 0.05$ ) with the statistical program Infostat.<sup>18</sup>

## Results and discussion

The results obtained in the two varieties of Swiss chard tested, the highest yields, expressed in fresh weight and number of leaves, were achieved with the inoculation of the microbial consortium of *A. brasilense* and mycorrhizal fungi, registering highly significant differences, with a p-value of 0.0001 for the number of leaves and a p-value of 0.0008 for the fresh weight of Penca blanca chard leaves and a p-value of 0.0002 for the amount of leaves and 0.0021 for fresh weight of chard leaves of the Arco iris variety (Table 1).

In the two varieties of Swiss chard evaluated, the application of the microbial consortium produced results that exceeded the control treatment, results consistent with those obtained by Ardisana et al.<sup>19</sup> who studied the effect on the growth of chard (*Beta vulgaris* L. subsp. *cicla*) plants of two biostimulants made with vermicompost leachates.

In the Penca blanca variety, 7 harvests were carried out, where an average production per plant during the entire crop cycle was 5,968.8 g in the control plants and 8,639.1 g in those inoculated with the microbial consortium (Table 2), while in the Arco iris variety, 6 harvests were carried out and an average yield per plant of 12,039.5 g in the control treatment plants and 16,997.5 g in the control plants inoculated chard plants (Figure 2, Table 3).

The Arco iris Swiss chard variety was more productive than the traditionally cultivated Penca blanca (Figure 3, 4). The total biomass accumulation of Penca blanca chard coincides with the results obtained by Barrientos Llanos et al.,<sup>15</sup> when growing Swiss chard in greenhouses, where the maximum total biomass accumulation value was 114.9 grams, corresponding to the Fordhook giant variety, which generates quite a few leaves, but its quality is essentially that of generating thick and succulent stems.

These results are consistent with those reported by Campos Martínez et al.<sup>21</sup> that evaluated the effect of mountain microorganisms on the production of Swiss chard in the Popayán plateau, a work that evidences the positive effects on the soil and chard plants, thanks to the action of microorganisms, which help to carry out the mineralization of organic matter faster.

As this trial was carried out on a fertile substrate and favorable results were obtained by the inoculation of the microbial consortium composed of *Azospirillum brasilense*, as it is a bacterium that fixes

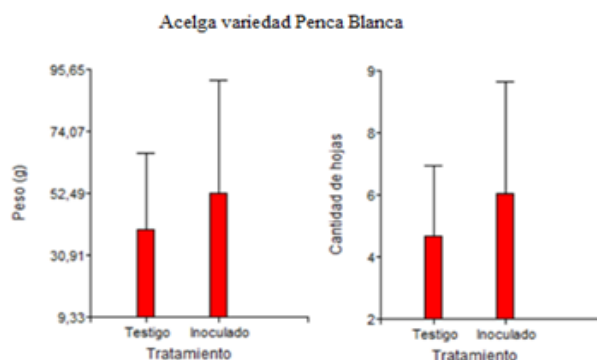
atmospheric nitrogen and mycorrhizal fungi, which allows us to infer that this biofertilizer will have a greater impact on soils of lower fertility.<sup>22,23</sup>



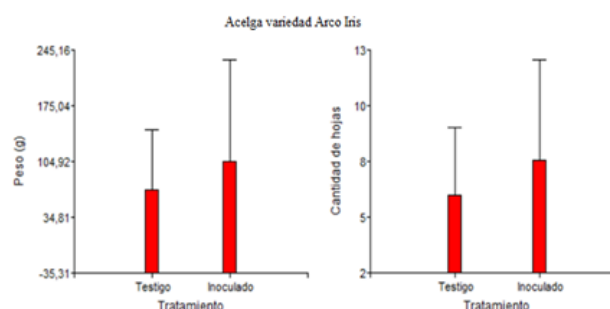
**Figure 1** Cultivation of two varieties of Swiss chard (*Beta vulgaris* L. subsp. *cicla*) Penca blanca and Arco iris, in a greenhouse with an agroecological approach.



**Figure 2** Average yield per chard (*Beta vulgaris* L. subsp. *cicla*) plant throughout the crop cycle.



**Figure 3** Fresh weight and number of leaves per chard (*Beta vulgaris* L. subsp. *cicla*) plant of the Penca blanca variety.



**Figure 4** Fresh weight and number of leaves per chard (*Beta vulgaris* L. subsp. *cicla*) plant of the Arco iris variety.

**Table 1** Comparison of fresh weight and number of leaves per plant of two varieties of Swiss chard (*Beta vulgaris* L. subsp. *cicla*) according to treatments

Cultivation	Treatment	Average sheet weight (g)	Average number of leaves (g)
White Chard Penca Blanca	Witness	39,72 ± 26,54 <b>A</b>	4,55 ± 2,10 <b>A</b>
	Inoculated	52,68 ± 39,05 <b>B</b>	5,82 ± 3,29 <b>B</b>
Arco iris Swiss Chard	Witness	69,59 ± 75,70 <b>A</b>	5,85 ± 3,31 <b>A</b>
	Inoculated	104,92 ± 127,49 <b>B</b>	7,59 ± 4,90 <b>B</b>

Uncommon letters in the same variable denote significant differences according to LSD test (Minimum Significant Difference) for P<0.05.

**Table 2** Comparison of the agronomic parameters of Swiss chard (*Beta vulgaris* L. subsp. *cicla*) Penca blanca variety (fresh weight and number of leaves) produced in the FCA-UNCa greenhouse

Days after planting	Variable	Treatment	
		Witness	Inoculated
60	Fresh Weight (g)	48,92 ± 35,14 <b>A</b>	57,84 ± 34,41 <b>A</b>
	Number of Leaves	2,87 ± 1,01 <b>A</b>	3,80 ± 1,99 <b>A</b>
65	Fresh Weight (g)	38,80 ± 22,10 <b>A</b>	28,83 ± 15,07 <b>A</b>
	Number of Leaves	3,86 ± 2,03 <b>A</b>	3,55 ± 1,47 <b>A</b>
70	Fresh Weight (g)	28,90 ± 13,42 <b>A</b>	25,27 ± 11,79 <b>A</b>
	Number of Leaves	5,31 ± 2,02 <b>A</b>	5,27 ± 2,18 <b>A</b>
80	Fresh Weight (g)	54,46 ± 30,59 <b>A</b>	91,56 ± 42,26 <b>B</b>
	Number of Leaves	5,48 ± 2,19 <b>A</b>	9,42 ± 3,20 <b>B</b>
90	Fresh Weight (g)	41,48 ± 28,45 <b>A</b>	32,92 ± 18,12 <b>A</b>
	Number of Leaves	4,67 ± 1,63 <b>A</b>	3,83 ± 1,40 <b>A</b>
100	Fresh Weight (g)	24,72 ± 8,87 <b>A</b>	22,94 ± 14,37 <b>A</b>
	Number of Leaves	5,41 ± 2,18 <b>A</b>	4,95 ± 3,29 <b>A</b>
120	Fresh Weight (g)	31,76 ± 19,49 <b>A</b>	71,18 ± 32,51 <b>B</b>
	Number of Leaves	3,81 ± 1,83 <b>A</b>	6,38 ± 2,64 <b>B</b>

Uncommon letters in the same variable denote significant differences according to LSD test (Minimum Significant Difference) for P<0.05.

**Table 3** Comparison of the agronomic parameters of chard (*Beta vulgaris* L. subsp. *cicla*) variety Arco iris (fresh weight and number of leaves) produced in the FCA-UNCa greenhouse

Days after planting	Variable	Treatment	
		Witness	Inoculated
60	Fresh Weight (g)	56,00 ± 21,07 <b>A</b>	84,06 ± 46,25 <b>B</b>
	Number of Leaves	3,50 ± 1,25 <b>A</b>	5,72 ± 3,29 <b>B</b>
65	Fresh Weight (g)	23,92 ± 19,14 <b>A</b>	32,94 ± 12,74 <b>B</b>
	Number of Leaves	4,23 ± 2,77 <b>A</b>	5,25 ± 2,18 <b>A</b>
100	Fresh Weight (g)	126,45 ± 79,15 <b>A</b>	249,96 ± 205,55 <b>B</b>
	Number of Leaves	8,28 ± 2,55 <b>A</b>	13,08 ± 5,44 <b>B</b>
120	Fresh Weight (g)	123,34 ± 111,17 <b>A</b>	162,62 ± 135,08 <b>B</b>
	Number of Leaves	8,06 ± 3,36 <b>A</b>	11,02 ± 5,44 <b>B</b>
130	Fresh Weight (g)	50,17 ± 23,39 <b>A</b>	61,07 ± 34,36 <b>B</b>
	Number of Leaves	3,18 ± 1,47 <b>A</b>	5,11 ± 2,41 <b>B</b>
145	Fresh Weight (g)	31,07 ± 21,73 <b>A</b>	48,18 ± 42,68 <b>B</b>
	Number of Leaves	5,11 ± 2,38 <b>A</b>	5,22 ± 2,07 <b>A</b>

Uncommon letters in the same variable denote significant differences according to LSD test (Minimum Significant Difference) for P<0.05.

## Conclusion

Therefore, it is concluded that inoculation with the microbial consortium composed of the bacterium *Azospirillum brasilense* and native mycorrhizal fungi positively influences the cultivation of the varieties of Swiss chard (*Beta vulgaris* L. subsp. *cicla*) Penca blanca and Arco iris, improving yield, health and safety, fundamental characteristics to achieve the success of the crop. This shows the potential of the bacterial strain and mycorrhizal fungi tested as a biofertilizer for the agroecological production of chard crops.

In addition, the present work provides information on a non-traditional variety in the region (Arco iris), while highlighting that the deepening of knowledge on the subject would contribute to a better understanding of the processes involved in the production of Swiss chard (*Beta vulgaris* L. subsp. *cicla*) in greenhouses with agroecological management.

## Acknowledgments

None.

## Conflicts of interest

Authors declare that there is no conflict of interest.

## References

1. Caballero-Mellado J. *The genus Azospirillum*. In "Microbes Online". In: E Martinez-Romero, et al. editors. Univ. Nac. Autonomous Region of Mexico. 2002. p. 177–198.
2. Bashan L, Holguin G, Glick B. *Growth-promoting bacteria in plants for agricultural and environmental purposes*. In: Agricultural Microbiology: Fungi, bacteria, micro and macrofauna, biological control, plants - microorganisms. In: Ronald Ferrera-Cerrato, et al., editors. Publisher by Editorial Trillas. Mexico. Chapter 8. 2007:170–224.
3. Di Barbaro Gabriela, Horacio Andrada, Valeria González Basso, et al. 2023. *Azospirillum* sp. and mycorrhizal fungi: key microorganisms in sustainable agriculture. *J Appl Biotechnol Bioeng*. 2023;10(6):199–204.
4. Kirk PM, Cannon PF, David JC. *Ainsworth and Bisby's Dictionary of the Fungi*. 9<sup>th</sup> edn. CAB International, Wallingford, UK. 2001;35(4):365–367.
5. Harrison MJ. Signaling in the arbuscular mycorrhizal symbiosis. *Annu Rev Microbiol*. 2005;59:19–42.
6. Selosse MA, Richard F, He X, et al. Mycorrhizal networks: des liaisons dangereuses?. *Trends Ecol Evol*. 2006;21(11):621–628.
7. Wang B, Qiu YL. Phylogenetic distribution and evolution of mycorrhizas in land plants. *Mycorrhizahello*. 2006;16(5):299–363.
8. Parodi G, Pezzani F. Arbuscular mycorrhizae on two grasses native to Uruguay in grazed and non-grazed areas. *Agrociencia Uruguay*. 2011;15(2):1–10.
9. Di Barbaro G, Andrada H, Gonzalez V, et al. Arbuscular mycorrhizae and dark septate fungi native to topinambur (*Helianthus tuberosus* L.) in Catamarca, Argentina. *Rev Cienc Agri*. 2017;34(2):98–106.
10. Newsham KK, Fitter AH, Watkinson AR. Multi-functionality and biodiversity in arbuscular mycorrhizas. *Trends in Ecology and Evolution*. 1995;10(10):407–411.
11. Pérez CA, Vertel MM. Evaluation of the colonization of arbuscular mycorrhizae in *Bothriochloa pertusa* (L) A. Camus grass. *MVZ Cordoba Magazine*. 2010;15(3):2165–2174.
12. Brundrett M. *Mycorrhizas in Natural Ecosystems*. In "Advances in ecological research". In: Begon M, et al., editors. *Academic Press Limited*. 2009;21:171–313.
13. Tarrand JJ, Krieg NR, Döbereiner J. A taxonomic study of the *Spirillum lipoferum* group, with descriptions of a new genus, *Azospirillum* gen. nov. and two species, *Azospirillum lipoferum* (Beijerinck) comb. nov. and *Azospirillum brasilense* sp. nov. *Can J Microbiol*. 1978;24(8):967–980.
14. Döbereiner J, Baldani VLD, Baldani JI. *How to isolate and identify diazotrophic bacteria from plants and legumes*. Brasilia: EMBRAPA-SPI. Itaguaí, RJ: EMBRAPA-CNPAB. 1995:11–60.
15. Manacorda AM, Tables DP, Álvarez AS. *Practical Manual of Microbiology - Volume I: Environmental Microbiology I. Ch 8: Counting Microorganisms*. 2007: p. 8.
16. Sieverding E. *Mycorrhizal Project*. International Centre for Tropical Agriculture. Cali. Colombia. 1983. p. 121.
17. McGonigle TP, Miller MH, Evans DG, et al. A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. *New Phytol*. 1990;115(3):495–501.
18. Di Rienzo JA, Casanoves F, Balzarini MG, et al. InfoStat version 2018. Grupo InfoStat, FCA, Univ. Nac. From Córdoba, Argentina. 2018.
19. Ardisana Eduardo Héctor, Antonio Torres-García, Osvaldo Fosado-Téllez, et al. Influence of biostimulants on the growth and yield of short-cycle crops in Manabí, Ecuador. *Tropical Crops*. Ministry of Higher Education. *National Institute of Agricultural Sciences*. 2020;41(4):e02.
20. Barrientos Llanos Harold, Carmen Rosa del Castillo Gutiérrez, Magali García Cárdenas. Analysis of functional growth, biomass accumulation, and dry matter translocation of eight greenhouse-grown vegetables. *Journal of Agricultural Research and Innovation and Natural Resources*. 2015;2(1):117–118.
21. Campos Martínez, Andrea del Pilar, Rosa Lina Acosta-Sánchez, et al. Evaluation of mountain microorganisms (MM) in chard production in the Popayán plateau. *Biotechnology in the Agricultural and Agroindustrial Sector*. 2014;12(1):79–87.
22. Topalović A, Knežević M, Trifunović S, et al. Effects of soil properties and fertilization on quality and biological activity of Swiss chard. *European Journal of Horticultural Science*. 2018;83(6):374–381.
23. White FA, Rooms EA. Mycorrhizae in Agriculture: Global Context and Research in Costa Rica. *Agron Costa Rican*. 1997;21(1):55–67.