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Mitigation of osmotic stress by *Serratia nematodiphila* in tomato seedlings

Mitigação de estresse osmótico por *Serratia nematodiphila* em plântulas de tomate

Mitigación del estrés osmotico por *Serratia nematodiphila* en plantas de tomate

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Tiandra Silva Carvalho
ORCID: https://orcid.org/0000-0003-4692-0737
Universidade de Vassouras, Brasil
E-mail: tiandra.carvalho@hotmail.com
Ana Carla Pinheiro Lima
ORCID: https://orcid.org/ 0000.0002-2926-6086
Universidade Federal Rural do Rio de Janeiro, Brasil
E-mail: carlabiomar@bol.com.br

Abstract

Agriculture is the main economic activity responsible for the highest rates of water consumption worldwide. Understanding strategies that combine decreasing the amount of water available and reducing the addition of chemical fertilizers is a major challenge today. Within this context, the work aimed to evaluate the efficiency in promoting growth in tomato seedlings, inoculated with *Serratia nematodiphila*, submitted to different water deficit conditions. The methodology consisted of using tomato seeds cv. Santa Clara Miss Brasil previously disinfected and inoculated with the *Serratia nematodiphila* bacterium. The experimental design was completely randomized in the factorial scheme 2 (with bacteria and without bacteria) x 3 (irrigation levels: 100%, 50% and 25% water), making a total of 6 treatments with 6 repetitions. When evaluating the effect of the bacteria *Serratia nematodiphila* in the treatment with irrigation to 25% of water, it was observed that the average values of the root length of the tomato seedlings was the one that suffered the most stimulus in the root growth when compared to the other irrigation levels. These results suggest that under conditions of greater water deficit the bacteria is able to mitigate drought by promoting root growth.

Keywords: Drought; Osmotic stress; *Serratia*; Tomato.

Resumo

A agricultura é a principal atividade econômica responsável pelos maiores índices de consumo de água em todo o mundo. Compreender estratégias que combinem a diminuição de água disponibilizada e a redução de adição de adubos químicos é um grande desafio nos tempos de hoje. Dentro desse contexto, o trabalho teve por objetivo avaliar a eficiência na promoção de crescimento em plântulas de tomate, inoculadas com *Serratia nematodiphila*, submetidas a diferentes condições de déficit hídrico. A metodologia consistiu em utilizar sementes de tomate cv. Santa Clara Miss Brasil previamente desinfestadas e inoculadas com a bactéria *Serratia nematodiphila*. O design experimental foi inteiramente casualizado no esquema fatorial 2 (com bactéria e sem bactéria) x 3 (níveis de irrigação: 100%, 50% e 25% de água), perfazendo um total de 6 tratamentos com 6 repetições. Ao avaliar o efeito da bactéria *Serratia nematodiphila* no tratamento com irrigação à 25% de água, observou-se que os valores médios do comprimento radicular das plântulas de tomateiro foi o que mais sofreu o estímulo no crescimento radicular quando comparado aos demais níveis de irrigação. Esses resultados sugerem que em condições de maior déficit hídrico a bactéria é capaz de mitigar a seca promovendo o crescimento radicular.

Palavras-chave: Estresse osmótico; Seca; *Serratia*; Tomate.

Resumen

La agricultura es la principal actividad económica responsable de las tasas más altas de consumo de agua en todo el mundo. Comprender las estrategias que combinan la disminución de la cantidad de agua disponible y la reducción de la adición de fertilizantes químicos es un desafío importante hoy en día. En este contexto, el trabajo tuvo como objetivo evaluar la eficiencia en la promoción del crecimiento de las plántulas de tomate, inoculadas con *Serratia nematodiphila*, sometidas a diferentes condiciones de déficit hídrico. La metodología consistió en utilizar semillas de tomate cv. Santa Clara Miss Brasil previamente desinfectada e inoculada con la bacteria *Serratia nematodiphila*. El diseño experimental fue completamente al azar en el esquema factorial 2 (con bacterias y sin bacterias) x 3 (niveles de riego: 100%, 50% y 25% de agua), haciendo un total de 6 tratamientos con 6 repeticiones. Al evaluar el efecto de la bacteria Serratia nematodiphila en el tratamiento con riego al 25% del agua, se observó que los valores promedio de la longitud de la raíz de las plántulas de tomate fueron los que sufrieron el mayor estímulo en el crecimiento de la raíz en comparación con los otros niveles de riego. Estos resultados sugieren que, en condiciones de mayor déficit hídrico, la bacteria puede mitigar la sequía al promover el crecimiento de las raíces.

Palabras clave: Estrés osmótico; Sequía; *Serratia*; Tomate.

1. Introduction

Agriculture is the activity responsible for more than 70% of current water consumption worldwide (WWF Brasil, 2013). For this reason, there are several proposals and strategies that have been studied to increase efficiency in the use of water by plants, in the search to find a balance between commercial activity and the environment.

Although there are numerous technologies available for the rational management of irrigation, the vast majority of producers still irrigate inappropriately. For 2025, the projection of scientists is that 3.3 billion people worldwide will not have water for irrigation (Coletti & Testezlaf, 2003).

The correct use of irrigation in order to efficiently manage water, fertilizers and other inputs, is essential for maintaining the food supply, in balance with its growing demand, guaranteeing the conservation of the environment (Coletti & Testezlaf, 2003).

The water content of the soil must be kept between certain specific upper and lower limits, among which water is not limited to the plant, while leaching is prevented Morgan et al. (2001), with the replacement of water to the soil by irrigation, in quantity and at the right time, decisive for the success of horticulture (Marouelli et al., 1996).

The tomato is a dicot, from the Solanaceae family, which stands out worldwide among cultivated vegetables, due to its fresh consumption, and above all, industrialized, because its production and use is considered universal (Camargo et al., 2007).

Tomato cultivation involves several cultural practices and among them is irrigation, which is present in 100% of commercial crops. Tomato is the second vegetable in economic importance in the world, being surpassed only by potatoes (Alves, 2006). According to Chaves (2009), Brazil accounts for 3% of global tomato production. In the Brazilian economy, tomato culture has stood out not only for its economic value, but also for being an activity that generates a large number of jobs.

The tomato fruit (*Solanum lycopersicon* L.) consists of 93% water, making it one of the vegetables most sensitive to stress due to excess or deficit of this component (Dorais et al., 2001).

Alvino et al. (1986) pointed out that abundant irrigation reduces yield and fruit quality, in addition to increasing production costs. According to Pulupol et al. (1996), the reduction in plant growth, productivity, fruit size and weight, in addition to the incidence of apical rot was related to the water deficit during treatments. Thus, it becomes essential to carry out the rational management of irrigation, in order to maintain favorable soil moisture and plant health conditions (Silva & Marouelli, 1998).

One of the alternatives that has been showing beneficial effects is the application of plant growth-promoting bacteria (PGPB) or plant growth-promoting rhizobacteria (PGPR). These bacteria facilitate the growth of plants, through any assistance in the acquisition of essential mineral resources, by modulating levels of plant hormones or even indirectly, growth can be promoted by decreasing the inhibitory effects of various pathogens that act as agents of biocontrol (Ahemad & Kibret, 2014).

The use of PGPBs, with the potential to achieve this objective, has been highlighted due to its easy applicability in treatments of seeds, roots and also in the aerial part of the plant. The productivity efficiency of these groups of microorganisms can be applied to the planting of crops, constituting an interesting alternative, to minimize the negative effects of the water deficit. Another strong point is the fact that these bacteria are native to soils or plants, not interfering with the ecological balance and, therefore, fully fitting into the reality of organic and sustainable agriculture (Lima et al., 2020).

Sustainable agriculture requires the use of strategies that allow an increase in food production without harming the environment and health, within the economic, social and political context of each region. Thus, this study aimed to evaluate the efficiency in promoting growth in tomato seedlings, inoculated with *Serratia nematodiphila*, submitted to different water deficit conditions.

2. Material and Methods

The research was based on laboratory analysis according to the methodology proposed by Pereira, Shitsuka, Parreira, & Shitsuka (2018).

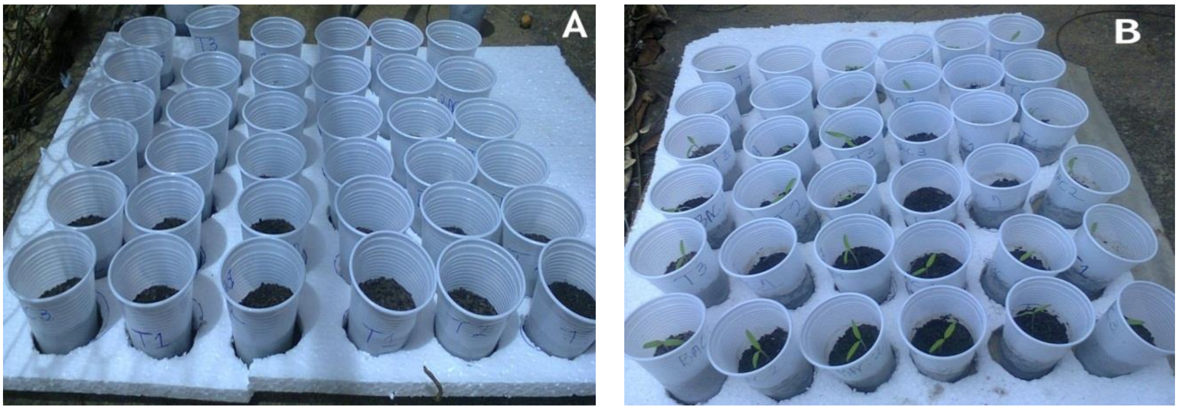
To evaluate the mitigation of osmotic stress in tomatoes, the endophytic bacterium *Serratia nematodiphila* was used, originally extracted from tomato seeds produced in the Horticulture sector at UFRRJ, Seropédica-RJ.

The experimental test was carried out in environmental conditions, in the city of Paty do Alferes, in the interior of the South Fluminense region of Rio de Janeiro, with Latitude: 22º 25 '43 "S Longitude: 43º 25' 07" W and altitude: 610m.

Tomato seeds, cv. Santa Clara Miss Brasil, superficially disinfected by immersion in 50% alcohol solution (30 seconds), 0.7% hypochlorite solution (three minutes) and five successive washes in sterile distilled water in a vortex shaker, with five water changes. The previously disinfected seeds were inoculated with the endophytic bacteria using the vacuum method described by Bashan & Assouline (1983). Half of the seeds were deposited in Erlenmeyer containing 20 ml of sterile distilled water and the other half deposited in a bacterial suspension of the isolate at a concentration of 10^8 CFU/mL. The flasks were placed in a desiccator coupled to the vacuum pump with the equivalent of 400 mm Hg, with slow release of air in three successive cycles lasting five minutes each cycle, interspersed with three minutes of rest. The seeds were placed to dry in a laminar flow chamber in ventilation, with a temperature of 35 ºC for 1 hour. The tomato seeds were sown directly in disposable 200 ml cups containing 100 ml of commercial substrate for the production of vegetable seedlings with a composition according to the packaging: pine bark, peat, vermiculite, simple superphosphate, postassium nitrate and products formulated by third parties (HT Tropstrate).

The experimental design used was the CRD (completely randomized design), in a factorial scheme of 2 (without bacteria, with bacteria) x 3 (irrigation levels 100%, 75% and 25%), totaling 6 treatments with 6 repetitions (Figure 1).

Figure 1. A completely randomized experimental design, containing tomato seeds sown in plastic bottles containing typical chemical fertilizer. **A:** seeds on your first day. **B:** Tomato seedlings on their 18th day.



Source: Authors.

Irrigation was performed by gravity. To determine the amount of water to be applied, 3 glasses were separated, three from each treatment. Each morning these glasses received an amount of water capable of promoting drainage. The drained water was collected and the difference calculated for each glass. The amount of water applied was equivalent to the difference in the average between the applied volume and the drained volume (retained

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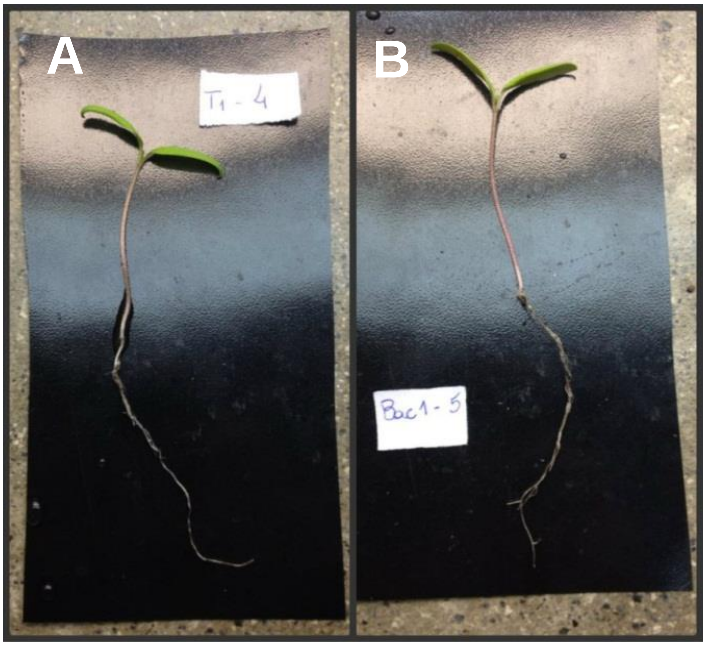
volume) in each treatment, multiplied by 100%, 50% or 25%, depending on the stress level of each treatment. After this procedure, the quantities of 35 ml of water for the treatment at 100%, 17.5 ml for the treatment at 50% and 8.75 ml of water for the treatment at 25% were determined.

The seeds were cultivated for 18 days, each one with its determined amount of water, until they reached a size sufficient to be measured. After these 18 days, the seedlings were removed from the cups and washed. After cleaning the seedlings, the length of the roots was measured with a digital caliper (Mitutoyo-Digimatic Calipter) (Figure 2).

Figure 2. A: Seedling without inoculation of bacteria, treatment at 100% water; **B**: Seedling with bacterial inoculation, 100% water treatment.



Source: Authors.

The data were organized in tables and the analysis of the results consisted of making basic statistics, using the Microsoft Office Excel ® spreadsheet for this purpose, and the different irrigation levels were adjusted according to the best equation for the coefficient, 2nd degree regression, tested by the corrected t test based on the residuals of the analysis of variance.

3. Results and Discussion

The data found in the present study revealed that the root length of tomato seedlings submitted to inoculation of the bacteria *S.nematodiphila* (T2) does not have a significant effect when compared to the control (T1), under conditions of 100% water irrigation (Table 1).

Table 1. Root length of tomato seedlings (mm) submitted to different levels of irrigation, without bacterization and bacterized, in the period from 4 to 22 September 2013.

	Root Length (mm)		
Treatments	**Average**	**Standard derivation**	**Variance**
T1	57,87	38,25	1463,18
T2	60,25	52,08	2712,96
T3	82,77	19,64	385,79
T4	93,35	17,33	300,34
T5	97,78	48,94	2395,29
T6	118,74	17,46	305,01

Legend: T1 (without bacteria + 100% irrigation), **T2** (with bacteria + 100% irrigation), **T3** (without bacteria + 50% irrigation), **T4** (with bacteria + 50% irrigation), **T5** (without bacteria + 25% irrigation), **T6** (with bacteria + 25% irrigation). Source: Authors.

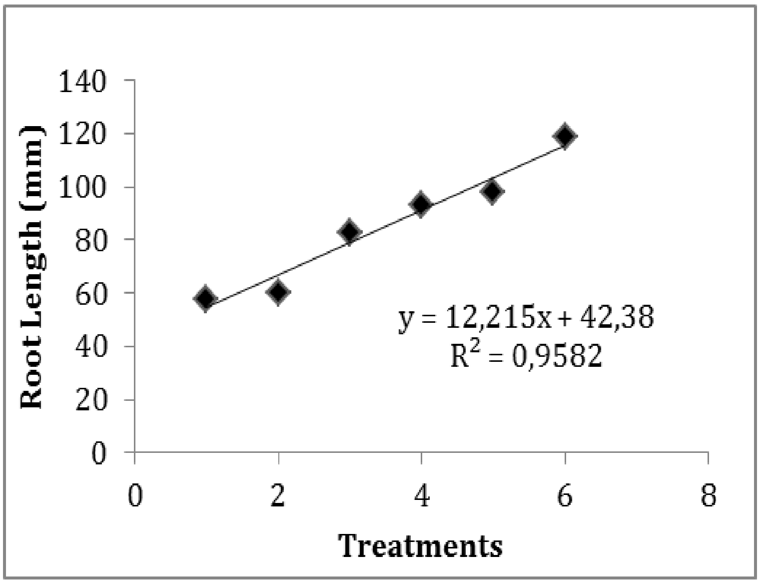
In treatment T4, irrigation at 50% water, the bacterium has a small effect on the promotion of root growth, when compared to treatment T2, although the average values recorded in table 1 do not differ significantly when compared to treatments T3 (control) and T4 (bacteria).

When evaluating the effect of the bacterium *Serratia nematodiphila* in the treatment with irrigation to 25% of water, it was observed that the average values of the root length of the tomato seedlings was the one that most suffered the stimulus in the root growth, reaching 118,74 mm (T6) when compared to other levels of irrigation. These results suggest that under conditions of greater water deficit, the bacteria is able to mitigate drought by promoting root growth (Table 1).

The positive effect of inoculation of the *Serratia* bacterium on the promotion of root growth was also observed in the work described by Lima et al. (2020) who, when evaluating the effect of osmotic stress imposed by PEG_{6000} at 7%, observed that tomatoes tend to grow more when inoculated with the bacteria, thus promoting a change in root architecture.

The regression analysis of the radicular length of the tomato during the imposition of different levels of irrigation showed a significant linear trend and an R^2 = 0.9582, revealing that the greater the water deficit (25% of irrigation), the greater the growth of the main root of the seedlings inoculated in relation to seedlings free from inoculation (figure 3). These results suggest that the promotion of axial root growth observed in tomatoes may be associated with the mitigation of water stress promoted by the inoculation of the bacteria *Serratia nematodiphila*.

Figure 3. Root length of tomato seedlings submitted to different levels of irrigation.



Source: Authors.

The results found in the study by Araújo et al. (2012), when verifying the efficiency in promoting root growth of the bacterium *Burkholderia* sp. inoculated in tomato seedlings submitted to different levels of water stress, corroborate the data found, when they reveal that the bacterium was also efficient in promoting root growth, however, the effect of the bacterium *Burkholderia* sp. was shown to be more efficient under adequate irrigation conditions, while the bacterium used in the present study, *Serratia nematodiphila*, showed more significant results in conditions where water stress was greater.

In the work of Medeiros (2013) similar results were observed when the bacterium *Herbaspirillum seropedicae* strain HRC54 was able to mitigate the stress imposed by polyethylene glycol (PEG_{6000}). According to the author, in the inoculated treatment + PEG, the growth of the axial root of the tomato was less reduced in response to the improvement of the presence of the inoculum when compared with the stressed non-inoculated plants.

The water stress mitigation process could also be associated with the availability and/or acquisition of resources essential to the plant, such as phosphorus and zinc for example. According to Rajkumar et al. (2010) the solubilization of inorganic minerals unavailable in the soil is one of the growth mechanisms promoted by bacteria of the genus *Serratia*. These microorganisms in situations of low iron availability in the environment are able to chelate and capture ferric ions, transporting the iron-siderophore complex into the cell.

4. Final Considerations

The results together allowed us to conclude that the bacterium *Serratia nematodiphila* is efficient in promoting root growth in plants subjected to water stress, presenting a significant result in the difference in the average length of plants inoculated with bacteria, from plants free from inoculation, with greater efficiency in conditions where water stress was greater.

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Percentage of contribution of each author in the manuscript

Tiandra Silva Carvalho – 50%

Ana Carla Pinheiro Lima – 50%