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Original article

The growth and physiological response of stevia (*Stevia rebaudiana* Bertoni) medicinal plant to inoculated with endophytic fungi and spraying of spermidine polyamine under salt stress conditions

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Extended abstract

Introduction

Salinity is one of the environmental abiotic stresses and most important factors limiting the growth and production of plants around the world, which dramatically affect various aspects of plant growth and development through anatomical, morphological and physiological changes (Siringam et al., 2011). Saline environments decrease the growth and yield characteristics of plants and increase some physiological properties such as proline content and electrolyte leakage (Noreen et al, 2010). On the other hand, in the recent years, the role of symbiotic fungi and polyamines in plants tolerance to environmental stress such as saline conditions are pronounced. Morever, due to the importance of medicinal plants, many researches of plant sciences have focused on the various aspects of the application of these plants (Vafadar et al., 2018). Therefore, the present study was conducted to investigate the growth and physiological response of stevia (Stevia rebaudiana Bertoni) medicinal plant to inoculation with endophytic fungi and foliar application of spermidine polyamine under salt stress conditions.

Materials and methods

This experiment was conducted at research greenhouse of Genetics and Agricultural Biotechnology Institute of Tabarestan (GABIT) at Sari Agricultural Sciences and Natural Resources University using factorial arrangement based completely randomized design with three replicates in spring and summer of 2016. The treatment consisted of salinity in three levels (0, 6 and 12 dS/m), fungal symbiosis treatments including four levels [non-inoculated (control), inoculation with Piriformospora indica (Pi), inoculated with Trichoderma virens (Trich) and co-inoculation of two fungi (Pi+Trich)] and foliar application of spermidine in three levels (0, 0.75 and 1.5 mM). Seedlings of stevia after inoculation with fungi transfered to adaptation chamber for 40 days and then moved to the greenhouse. Plants were irrigated with tap water until the end of vegetative stage and then irrigated with saline water treatments containing mixture of distilled and Caspian Sea water. The Spermidine was foliar applied one week before salinity stress. Two weeks after salinity stress, leaf samples were prepared to measure leaf relative

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water content (RWC), electrolyte leakage (EL), proline content and soluble sugars. Finally, the plants were removed from the pots and the growth and yield traits were measured.

Results

The result showed that in saline conditions, endophytic symbiosis, especially Pi+Trich, increased the stem dry weight (40-64%) and leaf dry weight (44-50%), relative water content (5-30%) and proline content (40-64%), and reduced EL (11-20%). Also spraying 0.75 mM spermidine signiticantly increased both leaf dry weight and plant height. Fungal symbiosis, especially Pi+Trich, and spermidine 0.75 mM resulted in an increase in the RWC. Also, spraying with polyamine spermidine at both concentrations of 0.75 and 1.5 mM increased soluble sugars and inoculated with endophytic fungi, particularly co-inoculation of two fungi, led to a reduction in the content of sugar (17%) in the stevia leaf. At the most levels of salinity and fungal treatments, spermidine, especially at the rate of 0.75 mM, led to increase in stem diameter (10-35%) and leaf area (35-46%).

Conclusion

In general, the results of the present study indicated a negative effect of salinity on the growth and physiological characteristics of the stevia plants. However, inoculation of endophytic fungi, particularly co-inoculation of Pi and Trich, improved the growth and physiological parameters and ameliorated adverse effects of salinity in stevia plants. Morever, the spermidine (especially 0.75 mM) induced salt stress tolerance in stevia plants and showed a synergetic effects with endophytic fungi in terms of the mentioned parameters.

Acknowledgements

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***Keywords*:** Electrolyte leakage, Endophytic symbiosis, Leaf dry weight, Proline, Saline water, Soluble sugar

Table 1. Comparison of mean interactions of salinity stress, fungus and spermidine polyamide spraying in stem diameter of stevia

Salinity ($dS.m^{-1}$)	Spermidine (mM)	Endophytic Fungus			
		Non-inoculated	*P. indica* (Pi)	*T. virens* (Trich)	Pi+Trich
0	0	2.345^{a-e}	2.396^{a-e}	2.583^{abc}	2.777^{ab}
	0.75	2.127^{b-e}	2.379^{a-e}	2.874^{abc}	2.871^{a}
	1.50	2.430^{a-e}	2.540^{a-e}	2.438^{a-e}	2.506^{a-e}
6	0	2.532^{a-e}	2.371^{a-e}	2.409^{a-e}	2.548^{a-d}
	0.75	2.455^{a-e}	2.688^{ab}	2.557^{a-d}	2.747^{ab}
	1.50	2.506^{a-e}	2.167^{b-e}	2.532^{a-e}	2.515^{a-e}
12	0	1.965^{cde}	2.229^{a-e}	2.157^{b-e}	2.298^{a-e}
	0.75	2.308^{a-e}	2.169^{b-e}	1.880^{de}	2.527^{a-e}
	1.50	1.869^{e}	2.602^{abc}	2.489^{a-e}	2.573^{abc}

The meanings of the similar letter(s) are not significantly different from the LSD test at the 5% probability level

Table 2. Comparison of the mean interactions of salinity stress, fungus and spraying of spermidine on the leaf area (cm^2) of the stevia plant

Salinity ($dS.m^{-1}$)	Spermidine (mM)	Endophytic Fungus			
		Non-inoculated	*P.indica* (Pi)	*T.virens* (Trich)	Pi+Trich
0	0	19.1^{d-i}	23.9^{a-f}	24.9^{a-e}	25.0^{a-e}
	0.75	22.2^{b-g}	24.3^{a-f}	22.7^{a-g}	30.1^{a}
	1.50	20.5^{b-i}	22.8^{a-g}	27.8^{abc}	28.2^{abc}
6	0	13.9^{i}	19.2^{d-i}	21.1^{b-i}	22.1^{b-h}
	0.75	20.6^{b-i}	24.5^{a-e}	21.4^{b-i}	30.1^{a}
	1.50	20.9^{b-i}	25.6^{a-d}	21.3^{b-i}	28.3^{ab}
12	0	14.3^{hi}	18.7^{d-i}	18.1^{d-i}	20.0^{d-i}
	0.75	17.6^{e-i}	19.9^{d-i}	23.0^{a-g}	24.2^{a-f}
	1.50	15.4^{ghi}	20.4^{c-i}	16.6^{f-i}	22.5^{a-g}

The meanings of the similar letter(s) are not significantly different from the LSD test at the 5% probability level

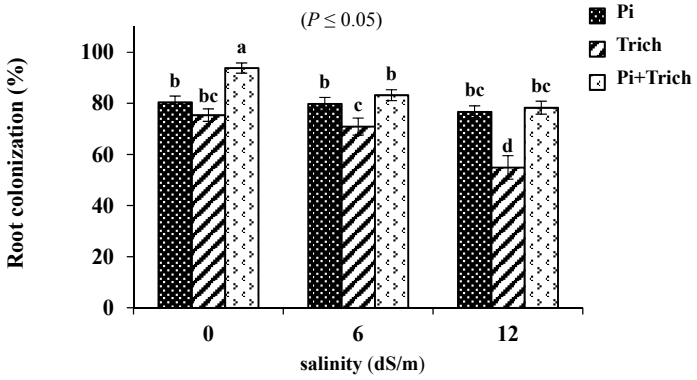

Fig. 1. Interaction between salinity and endophytic fungi on root colonization in stevia plant. In each figure, the meanings of the similar letter(s) are not significantly different from the LSD test at the 5% probability level. Vertical bars represent ±SE (n=9).

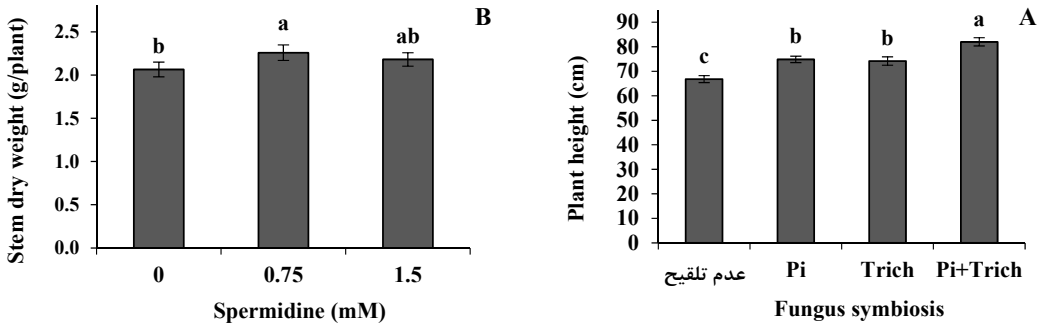

Fig. 2. The effect of fungi symbiosis on plant height (A) and the effect of spermidine polyamine on the stem dry weight of stevia (B). Pi: *P. indica* and Trich: *T. virens.* In each figure, the meanings of the similar letter(s) are not significantly different from the LSD test at the 5% probability level. Vertical bars represent ±SE (n=27).

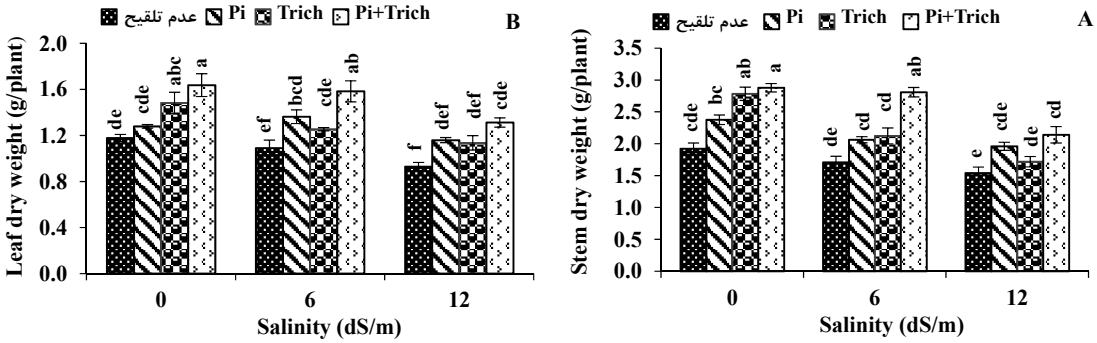

Fig. 3. Interaction of salinity and fungal symbiosis on stem dry weight (A) and leaf dry weight (B). Pi: *P. indica* and Trich*: T. virens*. In each figure, the meanings of the similar letter(s) are not significantly different from the LSD test at the 5% probability level. Vertical bars represent ±SE (n=9).

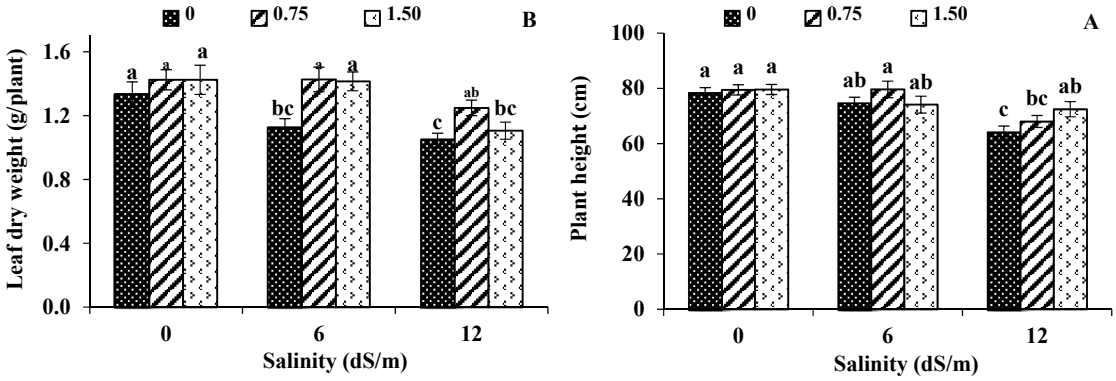

Fig. 4. Interaction of salinity and spermidine on plant height (A) and leaf dry weight (B). Pi: *P. indica* and Trich :*T. virens*. In each figure, the meanings of the similar letter(s) are not significantly different from the LSD test at the 5% probability level. Vertical bars represent ±SE (n=12).

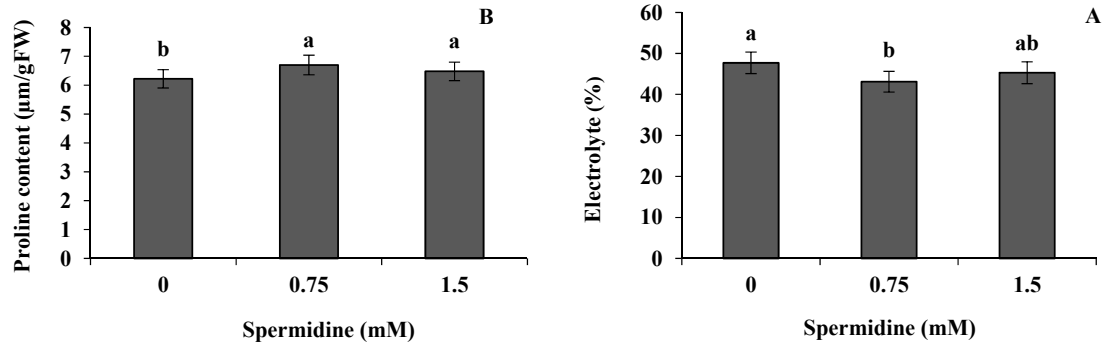

Fig. 5. Effect of spermidine spraying on electrolyte leakage (A) and proline content (B) in stevia leaf. In each figure, the meanings of the similar letter(s) are not significantly different from the LSD test at the 5% probability level. Vertical bars represent ±SE (n=36).

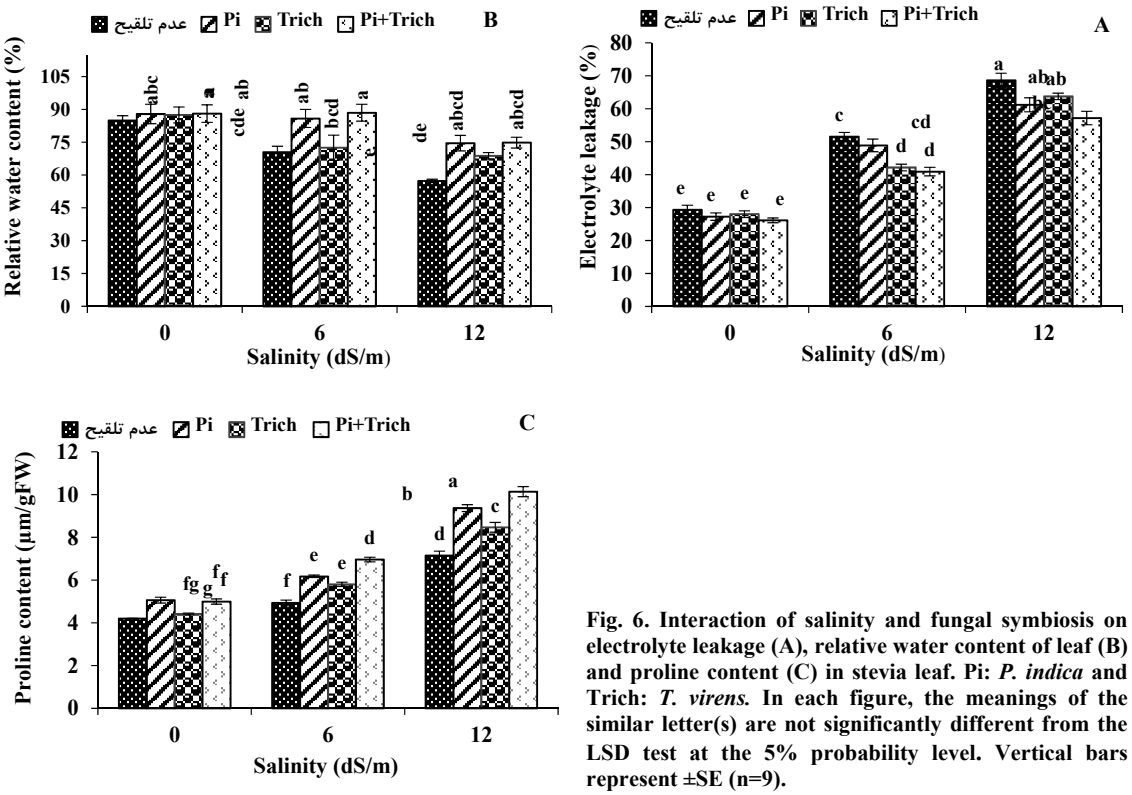

Fig. 6. Interaction of salinity and fungal symbiosis on electrolyte leakage (A), relative water content of leaf (B) and proline content (C) in stevia leaf. Pi: *P. indica* and Trich: *T. virens.* In each figure, the meanings of the similar letter(s) are not significantly different from the LSD test at the 5% probability level. Vertical bars represent ±SE (n=9).

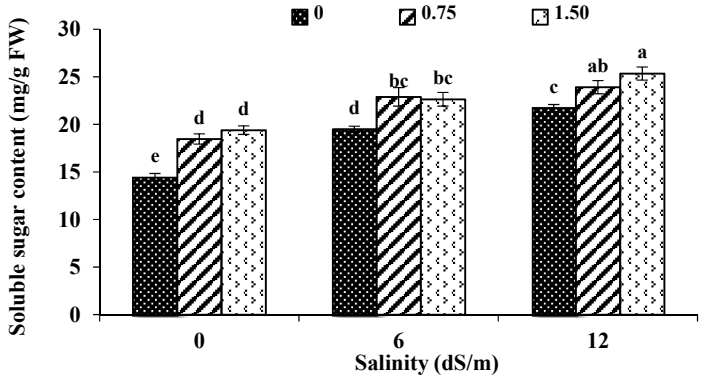

Fig. 7. Interaction of salinity and spermidine spraying on the content of soluble sugar in stevia plant. In each figure, the meanings of the similar letter(s) are not significantly different from the LSD test at the 5% probability level. Vertical bars represent ±SE (n=12).

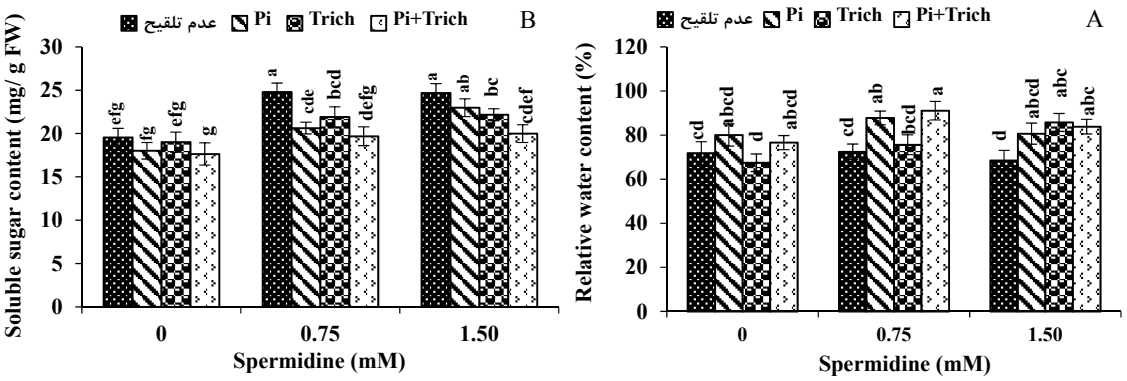

Fig. 8. Interaction of fungi symbiosis and spermidine spraying on the relative water content of leaf (A) and the content of soluble sugar (B). Pi: *P. indica* and Trich: *T. virens*. In each figure, the meanings of the similar letter(s) are not significantly different from the LSD test at the 5% probability level. Vertical bars represent ±SE (n=9).