

Effect of salt stress and soil amendments on *Stevia rebaudiana* Bertoni

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Outline

- Background
- Objectives
- Materials and Methods
- Results
- Conclusions
- Acknowledgement



Background



- *Stevia rebaudiana* Bertoni
- Steviol glycosides (SGs) are about 250-300 times sweeter than sucrose (Olsson et al., 2016), 43 identified (Gerwig et al., 2016)
- Major glycosides: stevioside, rebaudioside A (Reb A)
- Plants in nature are exposed to various environmental stressors, e.g. drought, extreme temperature, salt stress, etc.
- *Trichoderma atroviride*





Objectives

- To assess the effect of salt stress on the growth and steviol glycosides concentration in *S. rebaudiana*;
- To assess the impact of soil amendments (humic acid and *Trichoderma atroviride*) on responses of stevia plants to salt stress





Materials and Methods

- ▶ Plant growth condition
- ▶ Treatments
- ▶ Extraction of SGs
- ▶ HPLC analysis





Stevia plants grown in the research greenhouse at KPU





Materials and Methods - Treatment

Salt stress: 2 mM, 10 mM, and 30 mM NaCl

Soil amendments: Water

Trichoderma atroviride

Humic Acid (HA)



Materials and Methods - Extraction

Steviol glycosides (SGs) extracted using 60% ethanol Periche et al. (2015)



55°C for 15 min



5000g for 5 min



Materials and Methods – HPLC

Agilent 1260 infinity II HPLC system

Phenomenex Luna® HILIC column (Torrance, California)

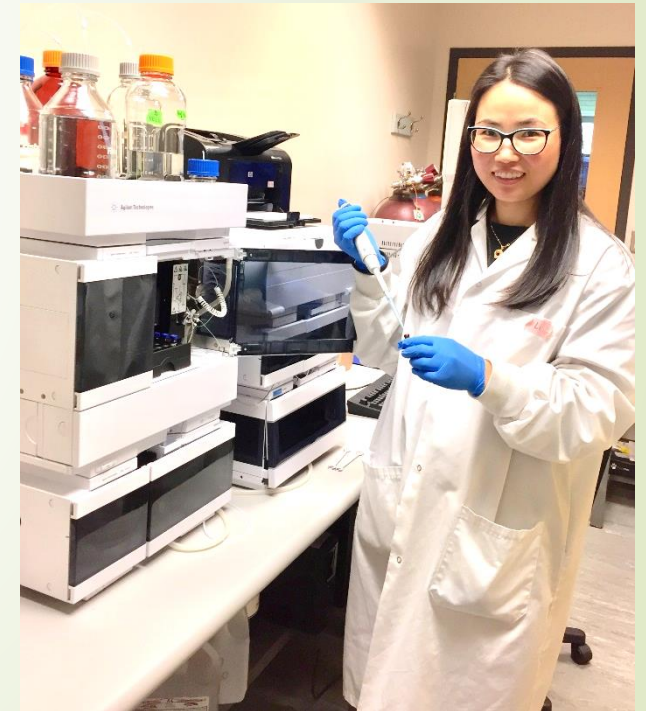
A: 0.01 mol/L phosphoric acid

B: Acetonitrile

Flow rate of 0.5 ml/min

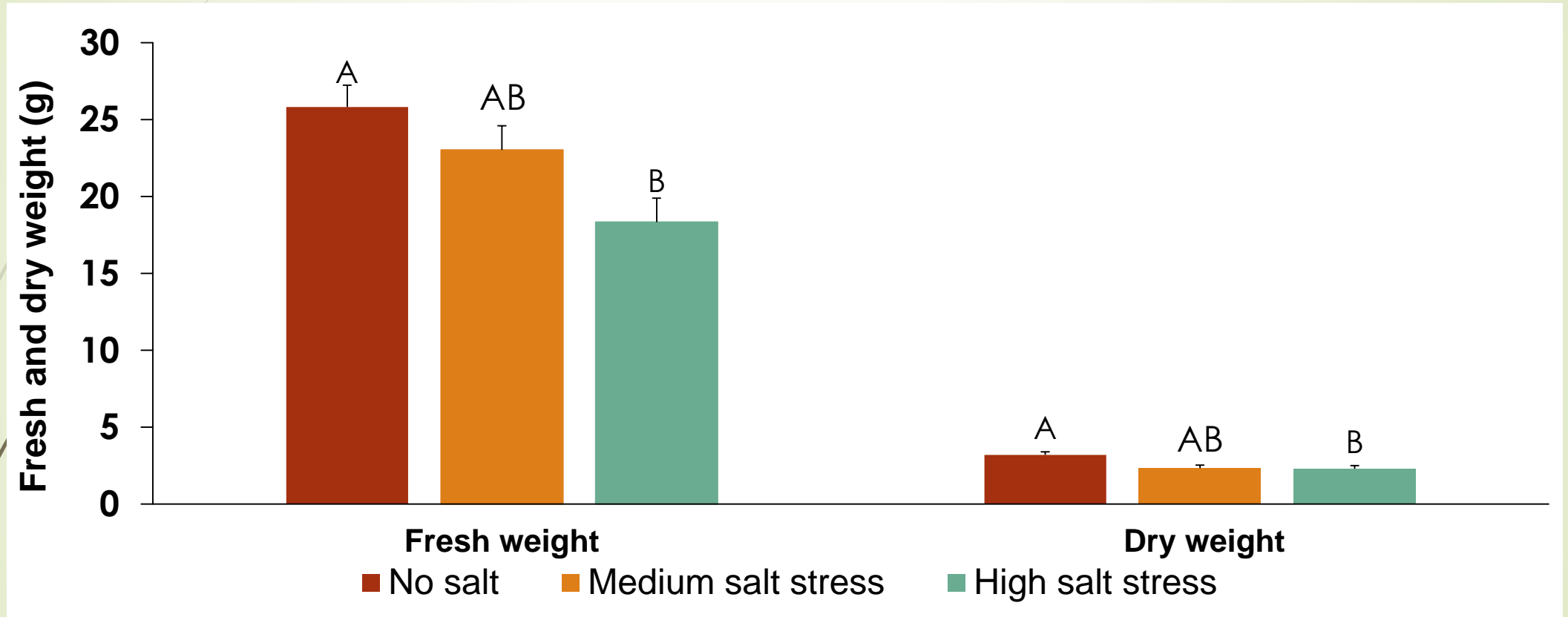
Injection volume was 5 μL

205 nm, 30°C



Results

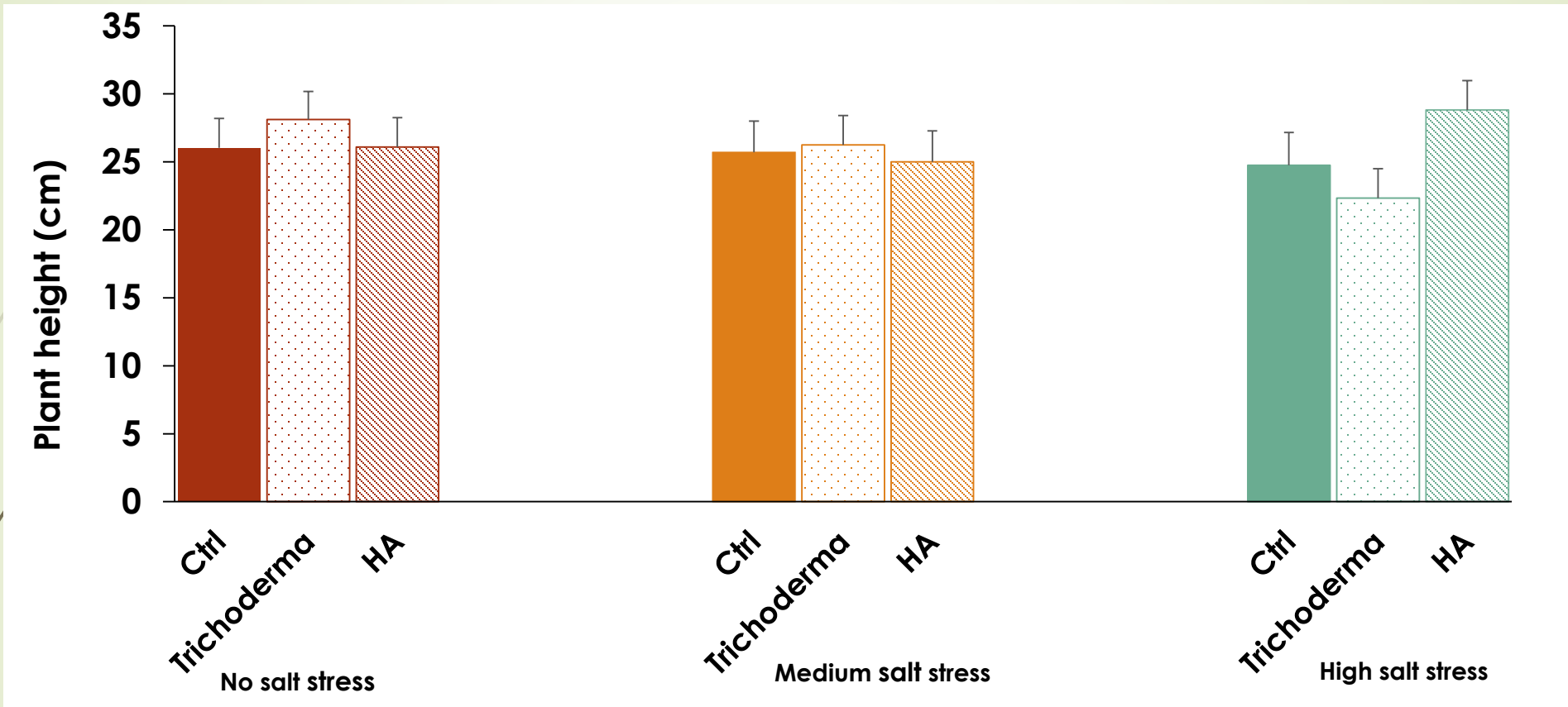
Both fresh and dry weights were affected by salt stress



Effect of salt stress on shoot weight of *Stevia rebaudiana* Bertoni. means \pm S.E. (n = 10).



Results



Effect of salt stress and soil amendments on plant height. Means \pm S.E. (n = 10)



Results

No salt stress



A: No salt stress
B: medium salt stress
C: high salt stress
1: Water
2: Trichoderma
3: Humic acid

Medium salt stress

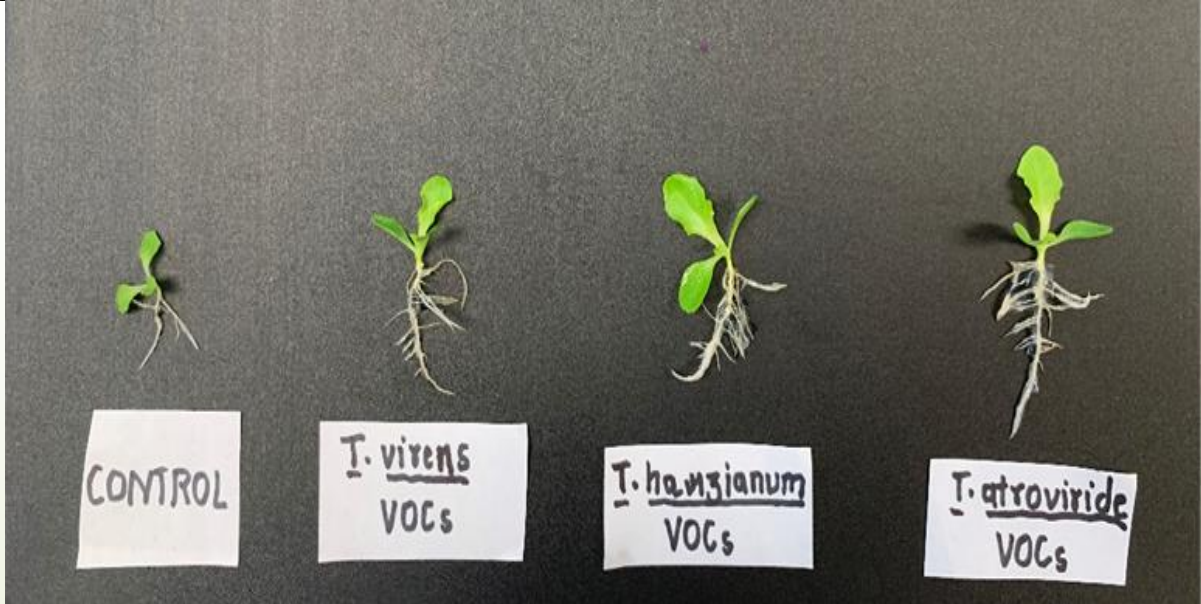
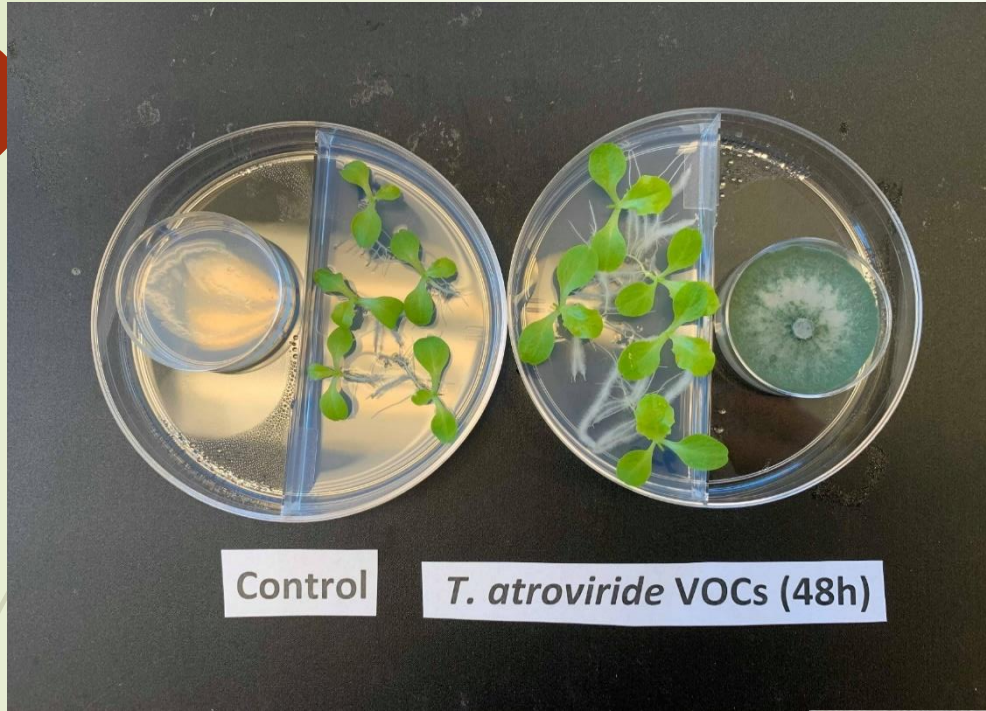


High salt stress



Effect of salt stress and soil amendments on roots of *Stevia rebaudiana* Bertoni





Results

Salt stress	Soil amendment	Stevioside (μg)	Reb-A (μg)	Reb-A/stevioside	RebA/(stevioside+ Reb-A)
Control	Water	11.46 \pm 1.88	4.01 \pm 0.86	0.36 \pm 0.05	0.26 \pm 0.03
	Trichoderma	12.51 \pm 1.82	2.94 \pm 0.36	0.23 \pm 0.04	0.18 \pm 0.02
	HA	15.34 \pm 1.00	2.99 \pm 0.59	0.20 \pm 0.03	0.16 \pm 0.02
Medium	Water	9.51 \pm 2.19	3.36 \pm 0.36	0.46 \pm 0.09	0.30 \pm 0.04
	Trichoderma	12.51 \pm 2.40	5.04 \pm 1.26	0.47 \pm 0.13	0.29 \pm 0.06
	HA	13.45 \pm 2.83	3.90 \pm 0.70	0.37 \pm 0.08	0.25 \pm 0.04
High	Water	15.63 \pm 2.89	3.51 \pm 0.64	0.32 \pm 0.07	0.23 \pm 0.04
	Trichoderma	12.84 \pm 3.00	4.93 \pm 0.86	0.48 \pm 0.08	0.31 \pm 0.04
	HA	13.70 \pm 2.28	3.04 \pm 0.49	0.29 \pm 0.06	0.22 \pm 0.04

Conclusions

- Salt stress affected plant growth as expected
- *Stevia rebaudiana* Bertoni is moderately salt stress tolerant
- Application of *Trichoderma* promoted root growth across all salt stress treatments
- Salt stress could be employed to modulate the ratio of glycosides in stevia plants



References

Gerwig GJ, et al. 2016. Stevia glycosides: chemical and enzymatic modifications of their carbohydrate moieties to improve the sweet-tasting quality. *Advances in Carbohydrate Chemistry and Biochemistry*. 73: 1-72.

Rodenburg DL, et al. 2016. Development of HPLC analytical techniques for diterpene glycosides from *Stevia rebaudiana* (Bertoni) Bertoni: Strategies to scale-up. *Journal of the Brazilian Chemical Society* 27: 1406-1412.



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Questions?

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