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Osmoprotective mechanisms of exogenous proline in salt-stressed *Physalis ixocarpa*: integrated morphophysiological, spectroscopic, and metabolomic analysis

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INTRODUCTION & AIM

The problem: soil salinization affects >800 million hectares globally, threatening crops like *Physalis ixocarpa* (Mexican husk tomato) in semi-arid regions.

The gap: while proline is a known osmolyte, its specific metabolic trade-offs and molecular mechanisms in *P. ixocarpa* remain unexplored.

Objective: to evaluate exogenous proline (seed priming and *in vitro*) as a salt stress mitigator using morphophysiological, ATR-FTIR, and GC-MS approaches.

METHODOLOGY

Screening: germination tests (0–200 mM NaCl) identified 75 mM NaCl as the threshold for moderate stress.

Treatments: *control*: no stress; *stress*: 75 mM NaCl; *proline*: 75 mM NaCl + proline (4, 6, 8, 10 mM).

Application: seed imbibition (30 min) for germination; media supplementation for *in vitro* culture.

Analyses:

- *Morphophysiology*: germination rate, biomass, chlorophyll (a/b).
- *Spectroscopy*: ATR-FTIR on roots, stems, leaves.
- *Metabolomics*: GC-MS of methanolic extracts.

RESULTS & DISCUSSION

A. Germination & growth restoration

Pretreatment with 8 mM proline was optimal, restoring germination to 78% (comparable to non-stressed levels) and recovering fresh weight (Table 1). Higher doses (10 mM) were counterproductive.

Table 1. Effect of proline pretreatment on germination (under 75 mm NaCl)

Treatment	Germination rate (%)	Fresh weight (g)
Control (no salt)	98.0 ± 1.4 ^{a}	1.354 ± 0.026 ^{a}
75 mM NaCl	62.0 ± 0.8 ^{d}	0.642 ± 0.022 ^{c}
NaCl + 4 mM proline	70.7 ± 2.5 ^{c}	0.758 ± 0.015 ^{b}
NaCl + 6 mM proline	76.7 ± 0.9 ^{b}	0.799 ± 0.019 ^{b}
NaCl + 8 mM proline	78.0 ± 0.8 ^{b}	1.322 ± 0.022 ^{a}
NaCl + 10 mM proline	70.7 ± 2.1 ^{c}	0.664 ± 0.008 ^{c}

RESULTS & DISCUSSION (cont.)

B. The "resource reallocation" strategy

Proline-treated plants exhibited a strategic trade-off: they sacrificed root growth to protect the photosynthetic apparatus (Figure 1).

- **Root length:** reduced from 9.7 cm (control) to 5.1 cm (proline).
- **Chlorophyll:** increased significantly, exceeding even the non-stressed control.

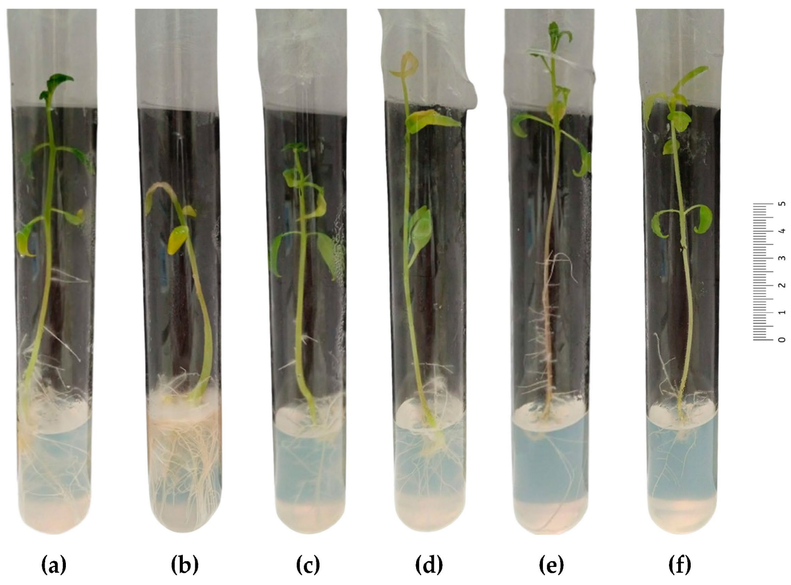

Figure 1. Visual comparison of seedlings. *Physalis ixocarpa* plants after 30 days of *in vitro* culture under different treatments: (a) control (no salt), (b) 75 mM NaCl, and 75 mM NaCl supplemented with (c) 4, (d) 6, (e) 8, or (f) 10 mM proline.

C. Metabolic & spectral reorganization

FTIR spectroscopy: Proline treatment restored polysaccharide bands (O-H stretching at 3400-3200 cm^{-1}) disrupted by salt, indicating cell wall stabilization.

GC-MS profiling:

- **Salt stress:** shifted dominant carbohydrate from *ethyl α-D-glucopyranoside* to *ethyl β-D-riboside*.
- **Proline restoration:** reversed this shift back to *α-D-glucopyranoside* (energy storage) and generated active pyrrolidine derivatives.
- **Mechanism:** phenolic antioxidants (e.g., catechol) disappeared under stress and were *not* restored by proline, suggesting proline acts via preemptive metabolic stabilization rather than antioxidant synthesis.

CONCLUSION

- **Optimal dose**: 8 mm proline is the most effective concentration for seed priming.
- **Mechanism**: proline operates through a "resource reallocation" strategy, prioritizing photosynthetic maintenance over root elongation.
- **Metabolism**: protection is achieved by stabilizing carbohydrate profiles and generating specific nitrogenous metabolites, reducing the need for costly phenolic antioxidant synthesis.