Gene expression and microbial interactions in Plantago spp under salt stress

IECAG 2024 Conference

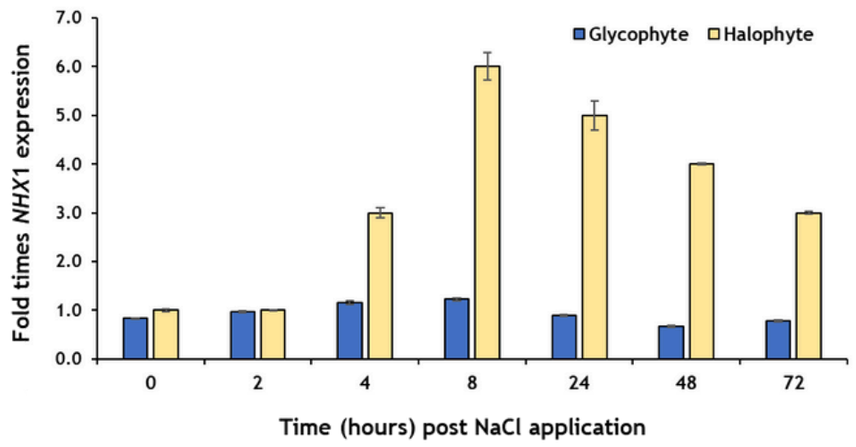
Silvia Potestio*, Antonio Vélez-Mejía, Monica Boscaiu, Giovanna Visioli, Oscar Vicente, Mario X Ruiz-González
Polytechnic University of Valencia
silvia.potestio@unipr.it

Salinity is a key abiotic stressor that impairs plant growth, reducing productivity and survival in natural ecosystems and agriculture. Understanding plant responses to salt stress is crucial for predicting ecosystem adaptation to climate change. Mediterranean coastal areas, like the salt marshes of La Albufera Natural Park in Valencia, Spain, face high salinity and host diverse plant species with varying salt tolerance.
For instance, species like Plantago coronopus and P. crassifolia exhibit greater tolerance compared to others such as P. major. Moreover, many Plantago species host microbial endophytes that may enhance salt stress tolerance.

EXPECTED OUTCOME

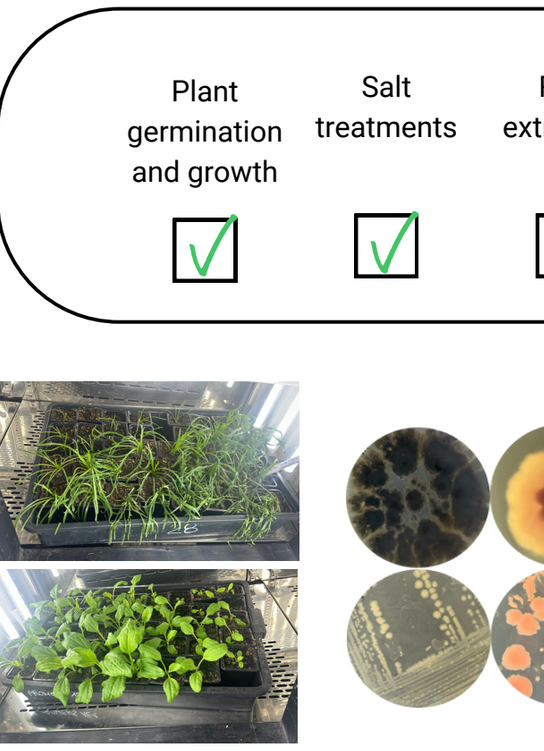
If halophytes (Plantago crassifolia) are actively transporting Na+ from the cytoplasm to the vacuole, we expect these plants to activate the expression of ion transport genes, such as NHX1.

HYPOTHESIS: soon after the application of salt, we expect the over-expression of NHX1 in halophyte plants



MATERIAL AND METHODS

Growth Chamber Pre-Experiments



- *Plantago major* (glycophyte) and *P. coronopus* (halophyte)
- Seeds were germinated and grown under controlled conditions in a growth chamber using sterile soil
- The plants were subjected to salt stress: 400 mM NaCl solution
- Samples were collected at six time points: 0, 2h, 4h, 8h, 24h, 48h, and 72h.
- RNA was extracted
- Quantitative real-time PCR (RT-qPCR) will be used to analyze the expression of the *NHX1* gene, a key regulator of sodium ion transport in plants.

Growth Chamber Experiments

- *Plantago major* (glycophyte), *P. coronopus* (halophyte), and *P. crassifolia* (halophyte).
- Seeds were germinated and grown under controlled conditions in a growth chamber using sterile soil
- The plants were subjected to salt stress: 400 mM NaCl solution
- Samples were collected at six time points: 0, 2h, 4h, 8h, 24h.
- RNA was extracted
- Quantitative real-time PCR (RT-qPCR) will be used to analyze the expression of the *NHX1* gene, a key regulator of sodium ion transport in plants.
- Biochemical analyses will be performed to evaluate stress markers, including proline levels, antioxidant activity, and other key metabolites.

PROJECT STATUS

Growth Chamber Pre-Experiments

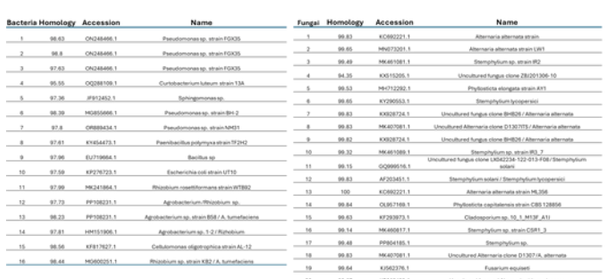
Plant germination and growth	Salt treatments	RNA extraction	NHX1 expression
☑	☑	☑	☐

Wild P. crassifolia

Endophytic bacteria and fungi	Mycorrhizal staining
☑	☑

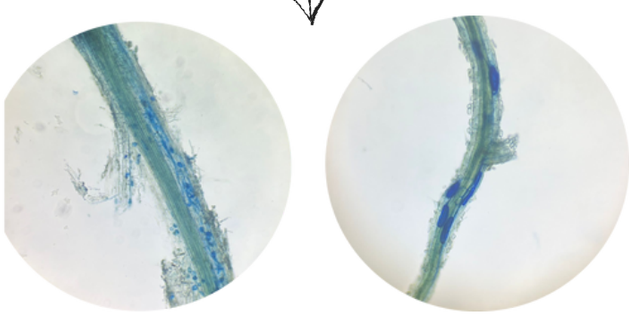
Growth Chamber Experiments

Plant germination and growth	Salt treatments	RNA extraction	NHX1 expression	Biochemical analysis
☑	☐	☐	☐	☐



Bacteria	Homology	Accession	Name
1	98.43	ON248466.1	Pseudomonas sp. strain FGX35
2	98.8	ON248466.1	Pseudomonas sp. strain FGX35
3	97.63	ON248466.1	Pseudomonas sp. strain FGX35
4	95.55	OQ288109.1	Curtobacterium luteum strain 13A
5	97.36	JF912452.1	Sphingomonas sp.
6	96.39	MG855666.1	Pseudomonas sp. strain BH-2
7	97.8	OR889434.1	Pseudomonas sp. strain NM31
8	97.61	KY454473.1	Paenibacillus polymyxa strain TF2H2
9	97.96	EU719664.1	Bacillus sp
10	97.59	KP276723.1	Escherichia coli strain UT10
11	97.99	MK241864.1	Rhizobium rosettiformans strain WTB92
12	97.73	PP108231.1	Agrobacterium-Rhizobium sp.
13	98.23	PP108231.1	Agrobacterium sp. strain BS8 / A. tumefaciens
14	97.81	HM153906.1	Agrobacterium sp. 1-2 / Rahnella
15	98.58	KF817627.1	Cellulomonas oligotrophica strain AL-12
16	98.44	MG660251.1	Rhizobium sp. strain KB2 / A. tumefaciens

Fungal	Homology	Accession	Name
1	99.83	KC692221.1	Alternaria alternata strain
2	99.66	MN073301.1	Alternaria alternata strain LW1
3	99.49	MK461681.1	Stemphylium sp. strain IR2
4	94.35	KX615205.1	Uncultured fungus clone ZB/2013/06-10
5	98.53	MH712292.1	Phyllosticta elongata strain AY1
6	99.65	KY290553.1	Stemphylium lycopersici
7	99.83	KX828724.1	Uncultured fungus clone BH826 / Alternaria alternata
8	99.83	MK407081.1	Uncultured Alternaria clone D1307T3 / Alternaria alternata
9	99.82	KX828724.1	Uncultured fungus clone BH826 / Alternaria alternata
10	99.32	MK461689.1	Stemphylium sp. strain IR3_7
11	99.15	OQ999516.1	Uncultured fungus clone LM042234-122-013-F08 / Stemphylium solani
12	99.83	AF203451.1	Stemphylium solani / Stemphylium lycopersici
13	100	KC692221.1	Alternaria alternata strain ML356
14	99.84	OL957169.1	Phyllosticta capitalensis strain CBS 128856
15	99.83	KF293873.1	Cladosporium sp. 10_1_M13F_A1i
16	99.14	MK460817.1	Stemphylium sp. strain CSIR1_3
17	99.48	PP804185.1	Stemphylium sp.
18	99.83	MK407081.1	Uncultured Alternaria clone D1307-A. alternata
19	99.64	KY562376.1	Fusarium equiseti
20	99.67	KP063439.1	Uncultured fungus / Alternaria chlamydospora
21	99.67	KP063439.1	Uncultured fungus / Alternaria chlamydospora
22	99.82	AJ300335.1	Cladosporium cladosporioides



FOLLOW UP

In the pre-experiment phase, RNA extraction has been completed, and RT-qPCR analysis will be performed to assess the expression of the NHX1 gene, providing insights into its role in salt stress response.
For the main experiment, RNA extraction and RT-qPCR for NHX1 will be carried out, alongside biochemical analyses to evaluate stress markers.
These investigations aim to unravel the molecular and biochemical mechanisms underlying salt tolerance in halophytes compared to glycophytes.

Funding for the experimental work was provided by Universitat Politècnica de València to Dr. Mario X. Ruiz-González: “Financiado con Ayuda a Primeros Proyectos de Investigación (PAID-06-22), Vicerrectorado de Investigación de la Universitat Politècnica de València (UPV)” and “Financiado con la Ayuda para potenciar la investigación postdoctoral de la UPV (PAID-PD-22)”.