SEARCHING FOR PGPM INDUCERS OF SALT TOLERANCE: FAST IN VITRO SELECTION AND EVALUATION IN TOMATO CULTURE

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Introduction and objectives

Many soils around the world are affected by excess salinity, caused by all mineral salts together affecting conductivity (saline soils) or sodium chloride (sodic soils). This affects 20-50% of irrigated farmland depending on the literature and globally represents approximately 60 million hectares worldwide. Soil salinization is a growing problem that threatens agricultural production and food security, since in saline soils, plant growth is impaired. Furthermore, it is exacerbated by climate change, which increases the likelihood of prolonged droughts. With the loss of arable land and population growth, the use of infertile land is becoming a major challenge. Conventional techniques for correcting saline soils, such as rinsing, leaching, and excavation (physical method) or the addition of gypsum-based soil improvers and lime (chemical method), have proven ineffective, costly, and unsustainable. The use of PGPM (plant growth-promoting microorganisms) can be an interesting complementary technique to the use of tolerant varieties to increase productivity in saline soil. This study aimed to test 23 bacterial isolates, 1 yeast isolate, and 1 fungal isolate.

Methodology

Screening Tests

Three in vitro screening tests were carried out to identify the best microbial strains exhibiting a growth-promoting activity under sodium salt stress conditions by direct and indirect mechanisms.

IAA Production test: The concentration of IAA produced by the strains was estimated using the Salkowski method. Candidate strains in pure cultures were incubated in the dark in liquid culture enriched with 200 mg.L^{-1} of L-tryptophan for 48 h at 150 rpm at 22°C (repeated twice). The supernatant was then added to Salkowski reagent (1:1) and ODs et 530 nm allowed to estimate the IAA concentrations produced.

Phosphorus solubility test (qualitative): Candidate strains were incubated as above in NBRI-BPB medium. In the presence of $Ca_3(PO_4)_2$ phosphorus, organic acids production will decrease the pH of their medium, causing the bromophenol blue to react and decrease in absorbance at 590 nm. The results are expressed as a percentage decrease in absorbance. This test provides results that correlate well with the values obtained with the quantitative test.

In vitro inoculation of tomato seedlings: After disinfecting tomato seeds of the 'Montfavet HF1' variety, 5 seeds were in vitro germinated on a MS agar medium with or without added salt (50 mm NaCl) (Fig.1). This medium is suitable for the growth of both the seedlings and the micro-organisms. This test allows a visual quick estimation of growth promotion properties through a few parameters: main root and stem length, secondary roots and leaves number.

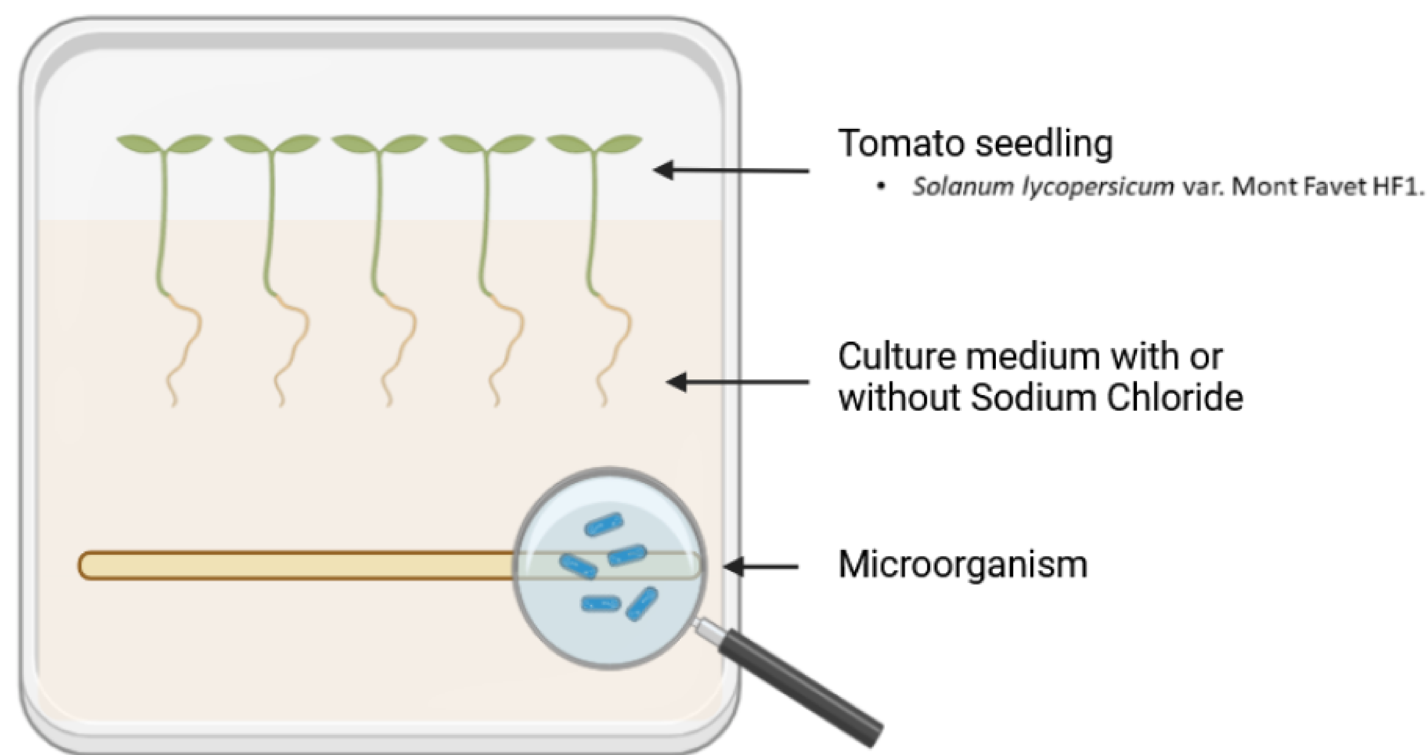

Figure 1 : ***methodology for in vitro inoculation testing of tomato seedlings.***

***In vivo* trial on tomatoes**: The trial, including 8 repetitions for each modality, was conducted in a greenhouse on *Solanum lycopersicum* var. Montfavet HF1. Plants were grown in 2 L pots with individual cups, in a medium-fertilized substrate (Klassman 1). The experimental design included 16 modalities, i.e. the 5 best strains selected from the 3 in vitro tests, two positive controls (a mineral fertilizer and the strain of a commercial microbial biostimulant product) and a negative control (Tab.1), with or without sodium chloride stress. Application of NaCl was carried out at sowing time at 200 mM NaCl and 2 weeks after sowing at 400 mM NaCl. During each application of saline solution, 50% of the useful reserve was filled (200 mL/pot). Microbial inoculations were carried out at the sowing. Measurements were taken during the trial (germination rate, chlorophyll content of leaves, the diameter of stems at the collar) and at the end of the trial after 6 weeks of cultivation (fresh and dry mass of aerial parts, the diameter of stems at the collar).

Table 1 : Inoculated strain and positive controls of the in vivo tomato trial.

Genus species	Strains	Source of isolation	Concentration of inoculation solution (CFU.mL^{-1})
Cryptococcus sp.	STSD4	Yeast isolated from tomato seeds variety Montfavet F1 (Botanic reseller) (TP 2021; L. Arminjon)	3,78E+06
Halomonas sp.	ESSD20	Bacteria isolated from *Salicornia europaea* (edible) (TP 2021; L. Arminjon)	3,70E+07
Paenibacillus sp.	1,2	Bacteria isolated from wheat seeds (TP 2018; F. Raffini)	2,90E+07
Gliomatix murorum	(4)10-1 iso 1	Fungus isolated from Algerian dune sand (L. Arminjon)	4,43E+05
Bacillus megaterium	MJ	Endophytic bacteria isoled from Horse chestnut tree (Maronnier Jacquet)	6,20E+04
Bacillus amyloliquefaciens	BA2 (C+)	Endophitycbacteria isolated from *Aquilaria crassna*	5,20E+05
Commercial fertiliser	C+	Universal fertilizer Wuxal G initial	20 g.L^{-1}

Results and Discussion

IAA production: the best performing strain was '*Bacillus sp.* strain 44' with an estimated concentration of 3.82 mg/L (Fig. 2), closely followed by '*Bacillus sp.* BCb1' with 3.61 mg/L.

Phosphorus solubilisation: the lowest absorbance value was observed for the '*Bacillus megaterium* MJ' strain, with a 41% decrease in absorbance (Fig. 2). The strains *'Bacillus amyloliquefaciens* 2330', *'Bacillus amyloliquefaciens* BA2', '*Paenibacillus polymyxa* BES 12' and '*Paenibacillus sp.* 1.2' ranked in second range an OD 590 nm decrease between 25% and 30%.

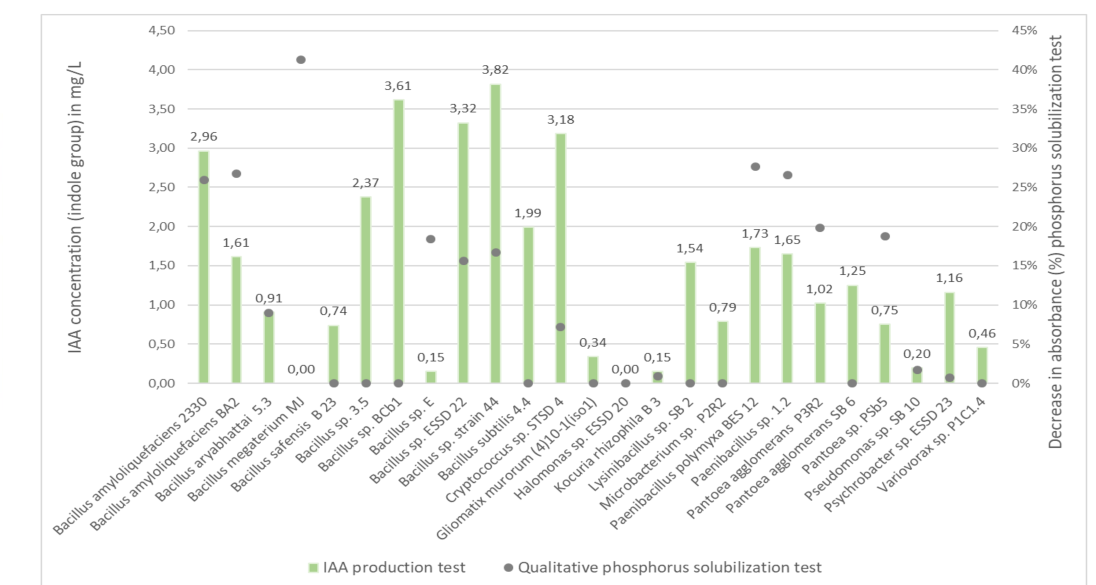

Figure 2 : ***In vitro screening tests for P and IAA on 25 microbial strains.***

Figure 3 : ***In vitro seedling inoculation test. The 'Cryptococcus sp. STSD4' strain (top right) and the 'Gliomatix murorum (4)10-1(iso1)' strain (bottom) showed the best growth promotion results on saline media after 4 weeks***

In vitro inoculation of tomato seedlings:
On saline medium, two strains showed strong growth promotion activity (Fig.3). The strains *'Bacillus megaterium* MJ', *'Paenibacillus sp.* 1.2' and *'Halomonas sp.* ESSD 20' also promoted seedling growth, but to a lesser extent.

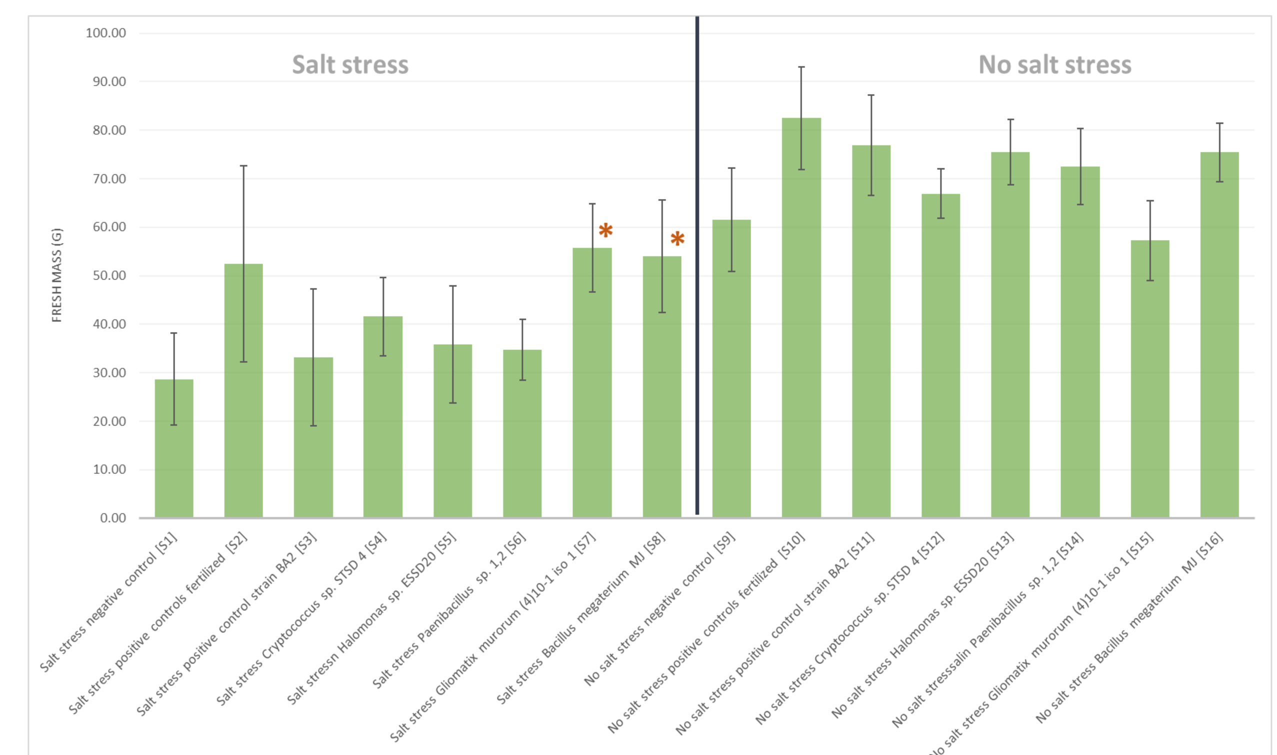

Figure 4: In vivo test: Average fresh mass of the aerial parts of tomato plants after 6 weeks of cultivation

Figure 5: Comparison between the negative controls and the two most effective treatments.

In vivo trial on tomatoes
Salt had a deleterious effect on the growth of tomato plants (Fig.4). The difference in average above-ground biomass was statistically significant (61.56 g for unstressed plants compared with 28.67 g for plants with salt stress). Under salt stress conditions, 2 treatments, *'Bacillus megaterium* MJ*'* and *'Gliomatix murorum* (4)10-1(iso1)*'* promoted statistically significant growth increases In particular, these treatments increased **fresh above-ground biomass** by 88% (54.01 g/plant) of the negative control value (28.67 g) for the *'*MJ*'* bacterial strain and up to 94% (55.74 g/) for the fungal strain *'*(4)10-1(iso1)*'*. The **dry above-ground biomass** of the plants was also similarly increased with these 2 treatments. Treatment with the 'MJ' strain resulted in significantly higher mean **collar diameters** than the negative control. There was no difference in mean **chlorophyll content.**

Conclusion

In this study, we demonstrated that particular microbial strains can increase plant biomass production under conditions of severe salt stress. The in vitro screening tests proved effective in quickly identifying the best-performing strains. The two best-performing strains need to be further tested under real conditions, in situ, using a suitable formulation and a standardized CFU concentration.