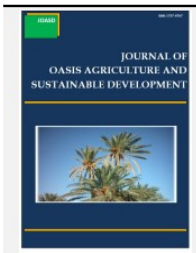

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Effect of NaCl and Polyethylene glycol on the in vitro growth of two potato fungal pathogens *Rhizoctonia solani* and *Fusarium solani*

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Abstract

Soil salinization and drought can adversely affect microorganisms inhabiting this compartment and modulate their interaction with plants. In this study we evaluated the tolerance of two soil born fungal strains belonging to the species *Rhizoctonia solani* and *Fusarium solani* to grow at different concentrations of salt (0 to 1000 mM of NaCl) and polyethylene glycol 6000 (0 – 37 % of PEG6000) in PDA and LB media. The results showed that *R. solani* showed better tolerance to NaCl concentration in LB in comparison to PDA medium, whereas the opposite behavior was noticed for *F. solani*. In addition, *F. solani* showed better tolerance to both NaCl and PEG6000 concentrations in comparison to *R. solani*. These results may help to understand and predict the impact of soil and water salinization on the development soil born fungal pathogens on cultivated plants.

1. INTRODUCTION

Extreme environmental conditions such as salinity, drought, high temperatures, and nutrient poor soils in arid and semiarid ecosystems affect plants production. In addition to these harsh environmental conditions, plants are subjected also to many diseases that reduce also their productivity and quality of their products. Researchers are no moving to decipher the mechanisms of tolerance of plants to combined stress i.e. abiotic and biotic stresses. In order to understand these mechanisms, it is mandatory to prospect the effect of the abiotic stress on the development of the pathogens.

Diseases caused by fungal pathogens are one of the most harmful diseases in plants causing yield reduction (Peng et al., 2021; Jain et al., 2019). Indeed, some of the world's great famines can be blamed on plant disease-causing fungi and fungal-like organisms such as the epidemic of *Tilletia* spp. on wheat, *Phytophthora infestans* on potato and *Plasmopara viticola* on vineyard. Soil born fungal diseases are very destructive with non effective control methods excepting genetic resistance (Iida, et al., (2022); Panth, et al., 2020). The effect of these soil born diseases is influenced by the soil conditions. Soil salinity and water availability can affect microorganism development and modulate their interaction with plants (Abdul Rahman et al, 2021). Fungal growth in the soil is influenced by various factors including salinity and drought which are major abiotic stress in arid and semiarid ecosystems (Gamalero et al., 2020). Salinity restricts fungal mycelium growth by the direct toxic effect of salts, limiting water uptake, modifying enzymes secretion to exploit the different food sources and through reducing carbohydrates availability (Asghari et al., 2008; Waheed et al., 2019). Several studies showed that fungal growth is affected with different manner depending from the fungal species, the levels of the constraints (Maharshi et al., 2021; Ritchie et al., 2006; Qiang et al., 2019).

So, this study aims to evaluate the effect of different concentrations of salt (NaCl) and Polyethylene glycol 6000on the growth of five

strains belonging to four soil born fungal pathogens.

2. MATERIAL AND METHODS

2.1. Fungal material

The fungal strains FS of *Fusarium solani* and RS5.2 of *Rhizoctonia solani* were used in this study (Djébali et al., 2014).

2.1. In vitro growth assay

The fungal strains were grown on Potato Dextrose Agar (PDA) and Luria Bertani Agar (LB) media supplemented with iso-osmotic concentration of NaCl (100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000 mM) and polyethlen glycol6000 (PEG) (13,19, 23, 27, 29, 31, 33, 35, 36, 37 %) in 90 mm Petri dishes.
Control PDA and LB media were not amended with NaCl and PEG6000. The Petri plates were incubated at 25 °C in darkness. The mycelia growth was assessed daily by photographing the fungal colony until it completely colonize the entire medium (from 4 to 12 days depending from the fungal species). The area of the fungal colony was estimated based on the photos using ImageJ software. At the end of the test, the AUGPC (Area Under the Growth Progress Curve) was calculated by the R statistical software to compare the growth of the different fungal strains. The experiment was repeated twice with three replicates per Treatment.

2.2. Medium preparation

The required NaCl quantities were added to the PDA and LB media before autoclaving. Whereas the PEG6000 was added to the media after autoclaving them since it prevents their solidification. So, solutions of 20 ml containing the required quantities of PEG6000 were autoclaved then poured on the solid PDA and LB media in 90 mm Petri dishes. The PEG6000 solution was left in contact with the medium overnight in order that the PEG6000 infiltrate into the solid medium. The following day, the excess of water was removed and the plates were dried under the laminar flow hood.

3. RESULTS AND DISCUSSION

Effect of the NaCl and PEG6000 on fungal growth: The growth variation depends of the fungal strain, the studied medium and the concentration of NaCl and PEG6000.
In free salt and PEG6000 PDA and LB media *R. solani* (RS5.2) strain showed a faster growth in comparison to *F. solani* (FS). The RS5.2 strain of *R. solani* completely colonizes the LB and PDA media at 4 and 6 days respectively. However, *Fusarium solani* (FS) it takes more than 10 days to colonize the entire medium. The strain RS5.2 of *R. solani* was very sensitive to NaCl and PEG6000 which decreased its growth since 100 mM of NaCl and 13% of PEG6000 in both media, in exception of 100 mM of NaCl on LB medium. The LB medium was more appropriate for the growth of RS5.2 in comparison to PDA medium in control and stressful conditions (Fig.1). The LB medium induced better growth of the studied fungal strains than the PDA medium due to its nutriment composition rich in sources of carbon and nitrogen. The concentration that inhibit 50% (EC50) of the *R. solani* growth is about 132 mM and 344 mM of NaCl in PDA and LB media

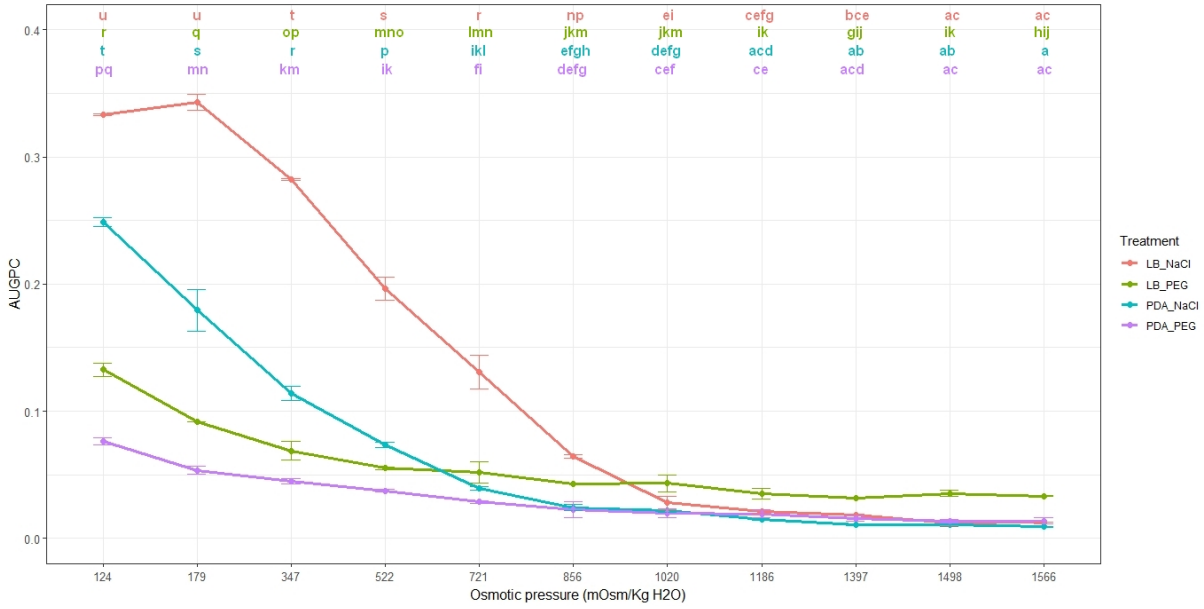

Fig. 1 Area under the growth progress curve (AUGPC) for the strain RS5.2 of *Rhizoctonia solani* cultured on Potato Dextrose Agar and Luria Bertani Agar amended with different concentrations of NaCl or PEG6000 at iso-osmotic pressures. Means indicated by the same letters are not statistically different according to Tukey's HSD test ($\alpha = 0.05$).

respectively, indicating that LB medium induce better tolerance to NaCl in comparison to PDA medium. The EC50 of *R. solani* growth on PDA and LB media amended with PEG6000 was about 20% and 17%, showing almost the same effect of both media amended with different concentration of PEG6000. Several previous studies showed that *R. solani* is sensitive to salt. Indeed, Ritchie et al. (2006) showed that the growth of all isolates of *R. solani* (AGs 2-1 and AG-3) generally declined with decreasing osmotic and matric potentials on PDA medium amended sodium chloride, potassium chloride, glycerol, and PEG6000.

In the soil, similar trend were also observed indicating that mycelia growth and sclerotia germination of *R. solani* AG-3 isolates declined with decreasing total water potential, with a minimum potential of -6.3 MPa permitting both growth and germination (Ritchie et al., 2006) which impact the behavior of R. solani in soil and its pathogenicity on solanaceous plants like potato. According to these results, the increase of soil salinity probably decreases the risk of infection with this fungal pathogen.

The *F. solani* (FS) showed better tolerance to NaCl and PEG6000 in PDA in comparison to LB medium (Fig.2). Indeed, the *F. solani* growth decreased significantly in comparison to the control at 500 mM of NaCl in PDA vs 300 mM in LB Medium. The growth of *F. solani* decreased significantly in comparison to the control at 19 % of PEG6000 in PDA and LB media, showing the same effect of both media amended with different concentration of PEG6000. The comparison between these two soil born fungi reveal that *F. solani* is more tolerant to NaCl and PEG6000 stress in comparison to *R. solani.* Haddoudi et al. (2021) showed an increase in growth rate of fungal strains under salt stress including different *Fusarium* species. Maharshiet al., (2021) observed also an increase of mycelial growth, mycelial biomass, sporulation, and microconidial production in Foc-49 strain of *F. oxysporum* cultured in high NaCl concentrations. However, Suwandiet al. (2018) showed that the mycelium growth of *Fusarium sp.* was decreased in response to increasing KCl concentrations. The capacity of adaptation of fungal species to water stress is due to the production of low molecular mass osmoregulatory substances to maintain the homeostasis of the intracellular space by preserving the cytoplasm water potential in comparison to the surrounding environment (Saito and Posas, 2012). Soil salinity is one of the most prominent abiotic stresses whereas wilt disease caused by *Fusarium* species is the major biotic stress in several cultures. The soil salinization increases the effect of disease wilting, which can be explained by the high tolerance of the *Fusarium* species to high salt concentrations.

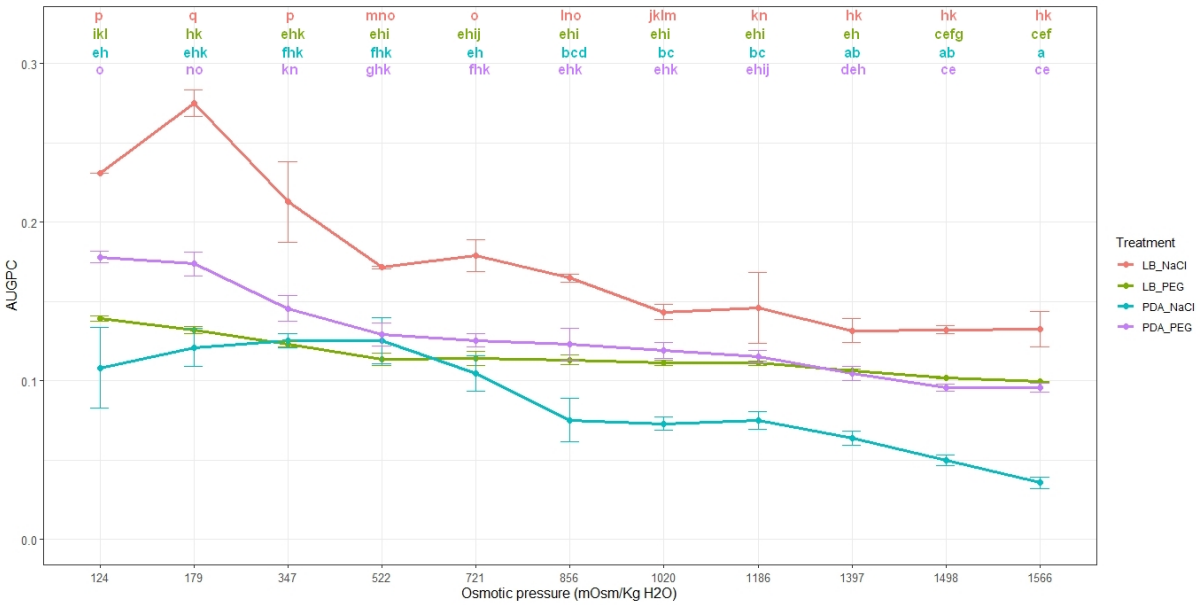

Fig. 2 Area under the growth progress curve (AUGPC) for the strain FS of *Fusarium solani* cultured on Potato Dextrose Agar and Luria Bertani Agar amended with different concentrations of NaCl or PEG6000 at iso-osmotic pressures. Means indicated by the same letters are not statistically different according to Tukey's HSD test (α = 0.05).

4. CONCLUSION

In conclusion, this study showed the different response of two soil born fungal pathogens to salt and osmotic stress. *R. solani* was very sensitive, whereas *F. solani* highly tolerant to salt stress which impact their pathogenicity on crops in soils affected with salt. These results may help to understand and predict the impact of salt and drought stresses on disease development on crops.

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