Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/jptcp.v30i1.2812

ISOLATION OF *GLOMUS ETUNICATUM* (*CLAROIDEOGLOMUS ETUNICATUM*) SPECIES ASSOCIATED WITH ROOTS OF (*DICHANTHIUM ARISTATUM*, BENTH) IN SALINE SOILS

Alexander Pérez Cordero[1*], Donicer E. Montes Vergara[2] , Yelitza Aguas Mendoza[3]

[1]*Universidad de Sucre, Facultad de Ciencias Agropecuarias, Colombia, https://orcid.org/0000-0003-3989-1747
[2]Universidad de Sucre, Facultad de Ciencias Agropecuarias, Colombia, donicer.montes@unisucre.edu.co, https://orcid.org/0000-0002-2860-0505
[3]Universidad de Sucre, Facultad de Ingeniería, Colombia, yelitza.aguas@unisucre.edu.co, https://orcid.org/0000-0003-4880-4510

***Corresponding Author:** Alexander Pérez Cordero
*Universidad de Sucre, Facultad de Ciencias Agropecuarias, Sincelejo, Sucre, Colombia, alexander.perez@unisucre.edu.co

ABSTRACT
The aim of this study was to isolate spores of arbuscular mycorrhizae-forming fungi from saline soils of cattle farms cultivated with Angletón grass in the municipality of Santiago de Tolú, department of Sucre, Colombia. Sampling, isolation, counting and identification of morphospecies were carried out on 35 cattle farms. A total of 120 morphospecies were isolated, of which the most predominant species corresponded to The morphospecies *Glomus etunicatum* Becker & Gerdermann, 1977, was reclassified as *Claroideoglomus etunicatum.* Therefore, these studies indicated that AMF (particularly *C. etunicatum*) has significant potential for improving the efficiency of the plant-microbe remediation of HM-contaminated soils.

Keywords: soil, salinity, mycorrhizae, pasture.

INTRODUCTION
Amongst the introduced species, the anglet grass (*Dichantium aristatum*, Benth) represents the third species with the largest sown area in the department of Sucre, reaching an area of approximately 56,200 hectares (ha), distributed in 19 municipalities. Sucre, reaching an area of approximately 56,200 hectares (ha), distributed in 19 municipalities. The municipality of Santiago de Tolú, whose main economic activity is semi-intensive livestock farming, has the second largest area planted with this species in the department, representing 9,400 hectares (Peroza and Pérez, 2010).

The soils of the municipality of Santiago de Tolú are located in the alluvial coastal plain sub-region or the Morrosquillo Gulf and are characterized by their flat topography, subject to flooding, chemically they have medium to high base saturation, their effective depth varies from shallow to moderate, limited by fluctuations in the water table, they have variable amounts of sodium, medium to high phosphorus levels, low to medium organic matter content, slightly acid to slightly alkaline pH and medium to high fertility. These soils are suitable for crops that tolerate the presence of sodium salts and flooding (Peroza and Pérez, 2010).

Salinity in natural (coastal) soils is a serious problem for soil health and plant growth and production (Azadi and Raiesi, 2021). More than 80% of coastal soils have soil with high potential for agriculture but are affected by salinity (Abdel Latef et al., 2020).

Soil microorganisms are an important part of the soil environment and soil process reactions. Their activity reflects the intensity and direction of various biochemical reactions (Xu et al., 2021). A slight decline in soil microbial diversity or changes in structure and function will affect the availability and absorption of nutrients by plants (Azarbad et al., 2015).

Soil worldwide has been severely damaged by various stresses, particularly in natural saline soils. The rhizosphere microbial community and arbuscular mycorrhizal fungi (AMF) can effectively facilitate the phytoremediation of contaminated soil.

Arbuscular mycorrhizae-forming fungi (AMF)can form symbiotic relationships with more than 80% of the plants on the planet (Zhang et al., 2018a). AMF are generally considered to improve plant tolerance to stressful environments, such as saline soils, and enhancing the ability of plants to remediate soil (Ma et al., 2019). Many studies have demonstrated that AMF alleviate the adverse effects of various stresses through mechanisms such as increasing nutrient absorption (particularly phosphorus [P]), maintaining ion balance, improving antioxidant systems, and protective enzyme activities (Hidri et al., 2019; Riaz et al., 2021).

Arbuscular mycorrhizal fungi play an important role in cycling and prevent fixation or washout; improve water relations; restore physical conditions and soil fertility in degraded ecosystems; improve aggregation and prevent erosion; stimulate plant growth and improve the nutritional quality of plant species. Additionally, mycorrhizal plants are more tolerant to adverse environmental conditions and are protected from root pathogens.

According to Barea eta al., (1999), drought and salinity are limiting factors in agricultural production, studies on mycorrhizae in these environments are relatively recent. Several authors point out that improved phosphorus nutrition in mycorrhizal plants is a key factor justifying increased tolerance to salinity conditions. The use of fungal isolates from saline environments that are more adapted to such stress conditions is considered of great interest. Recent studies corroborate that inoculation with mycorrhizal fungi increases plant tolerance to salinity.

Taking into account the benefits of this natural multifunctional symbiosis and the current state of soil microbiology in the Colombian Caribbean, there are no studies on this species of grass associated with these microorganisms, under this soil condition, it is proposed to isolate the different genera of native arbuscular mycorrhizal fungi associated with the Angletón grass species and their relationship with the physicochemical characteristics of the soil.

MATERIALS AND METHODS

Location. The study was carried out in the municipality of Santiago de Tolú, which is part of the alluvial coastal plain subregion or of the Gulf of Morrosquillo, it is located to the Northeast of the department of Sucre, it has an extension of 35750 hectares with 45 km of coast, located at an altitude that oscillates between 0 and 10 m above sea level. The vegetal formation corresponds to the tropical dry forest (*Holdridge Life Zone*), it is located in a dry warm climate, the materials that form the soils are constituted by alluvial sediments, marine or combinations of both. In some sectors there are organic sediments.

Sampling area. The sampling was carried out in the cattle farms established with Angletón grass in the municipality of Tolú, in agrological zones located according to the classification of the land by its capacity of use in the subclasses IIIsc and IVsc-Vhs.

Sampling. In each selected cattle farm, several sub-samples (soil and roots) were randomly collected at a depth of 0-20 cm, from which a sample of 2000 g was formed. The samples were placed in plastic bags labelled with the number of the farm, the area planted with Angletón and the date of collection. Once collected, they were refrigerated until use (Pérez and Peroza, 2013).

Isolation and identification of morphospecies. The collected samples were sieved to separate soil and roots. Once sieved, they were used to perform the following analyses: isolation of AMF spores, spore count (spores/100 g of soil), identification of morphotypes by shape, color and size of spores, following the methodology reported by Pérez and Vertel, 2010. The isolated spores were deposited in Petri dishes and the HMA-like characteristics were observed. Once the above process was completed, AMF morphotypes were identified using descriptive techniques proposed by INVAM, 2019.

RESULTS AND DISCUSSION

Figure 1 shows the number of livestock farms sampled and the number of morphospecies isolated on each farm. A total of 120 morphospecies were isolated under the soil conditions of cattle farms in the municipality of Santiago de Tolú.

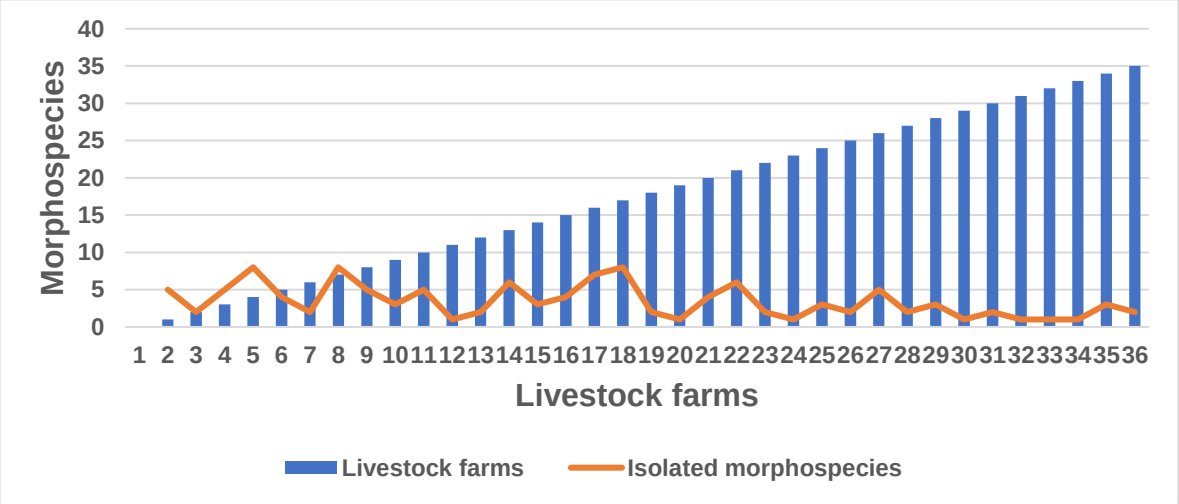


Figure 1. Number of morphospecies of arbuscular mycorrhizal fungi isolated from soils of cattle farms in the municipality of Santiago de Tolú, department of Sucre, Colombia.

According to the studies carried out by Pérez and Peroza, 2013, of the quantity of arbuscular mycorrhizal morphospecies in livestock soils in the municipality of Santiago de Tolú, the mathematical models built to evaluate the population density of AMF spores in soils of livestock farms in the municipality of Tolú according to physical-chemical parameters, indicate that the highest number of AMF spores in soils is directly related to the concentration of exchangeable elements such as magnesium (Mg), sodium (Na) and bulk density values (Da), and inversely related to the concentration of the exchangeable elements magnesium (Mg), sodium (Na), sodium (Na) and bulk density values (Da), sodium (Na) and bulk density (Da) values and inversely related to Copper (Cu) and Manganese (Mn) values and the models built to predict the number of spores as a function of salinity, shows that salinity interferes directly with sporulation in the presence of the variables pH, magnesium (Mg), sodium (Na), sulphate (SO4), percentage of exchangeable sodium (PSI) and the percentage of sodium in parts per thousand (S/00) and inversely proportional to electrical conductivity (EEC).

Specific studies on the presence of morphospecies of arbuscular mycorrhizal fungi in saline soil conditions in Colombia are scarce and inconclusive in relation to the influence of physical-chemical parameters and salinity on the in situ colonization of these mycorrhizae, associated to the rhizosphere and roots of Angleton grass. This makes it difficult to compare the results obtained in this study; however, the results obtained for the number of morphospecies are low.

Among the adverse conditions in pasture systems, salinity is the most important determinant of the establishment of microbial populations. Arbuscular mycorrhizal fungi protect plants from salinity stress, but the mechanisms involved are not well understood. This is partly because there are few studies on the effects of AMF on plant growth under salinity conditions and the effects of salinity on colonization by arbuscular mycorrhizae. However, available information indicates that these fungi have the potential to increase the benefits derived from salt tolerant crops when properly selected and combined (Azcón, et al., 1997). In saline soils mycorrhizae-forming fungi are considered to improve the supply of mineral nutrients to plants, especially the supply of phosphorus which tends to be precipitated by ions such as Ca_2^+, Mg_2^+ and Zn_2^+(Al-Karaki,2002; (Al-Karaki et al., 1998; Al-Karaki et al., 2001; Aliasgharzadeh et al., 2014). In addition to nutritional improvement, AMF benefit physiological processes such as plant water uptake capacity, increase root hydraulic conductivity and favors the adaptation of osmotic balance and carbohydrate composition ((Porras et al., 2009; Rosendahl and Rosendahl, 1991). In this way, these fungi attenuate the adverse effects of excess salt accumulated in the roots (Giri and Mukerji, 2003).

Glomus etunicatum Becker & Gerdermann, 1977, is one of the predominant morphospecies in the cattle-raising soils of the municipality of Santiago de Tolú. The main characteristics of the morphospecies are described in figure 2.

The morphospecies Glomus etunicatum Becker & Gerdermann, 1977, was reclassified as *Claroideoglomus etunicatum.* The beneficial effects of *C. etunicatum* on *Triticum aestivum*, *Zea mays*, and *Medicago sativa* growth under HM stress have been reported in previous studies, primarily reflecting improvement in plant nutrient status, enhancement in the activities of antioxidative enzymes and antioxidative molecules, decreases in HM uptake, and translocation in plants and plant organs (Baihui et al., 2022). Therefore, these studies indicated that AMF (particularly *C. etunicatum*) has significant potential for improving the efficiency of the plant-microbe remediation of HM-contaminated soils.

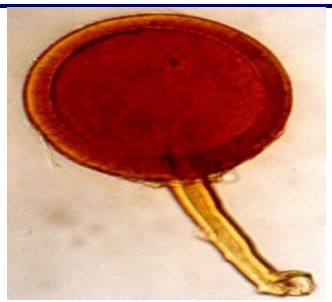
Morphospecies	40X
Glomus etunicatum Becker & Gerdermann, 1977.	
DESCRIPTION OF THE SPORE: Form: ▪ Globosa Diameter (µm): ▪ 75-87 Colour: ▪ water: Orange ▪ P.V.L: dark orange Cytoplasmic content: ▪ Granular Surface structure: ▪ smooth	
Composition and wall type:	Number of layers: ▪ Single layer with laminated appearance Wall width (µm): 7.8 - 9.9
Width and type of hyphal junction:	Hyphal diameter (µm):13.2 Pore diameter (µm): 3.9
Additional remarks: ▪ presence of channels in the wall	
Taxonomic determination: ▪ Genus: *Glomus* ▪ specie: characteristics similar to *Glomus etunicatum Becker & Gerdermann, 1977*. RECLASSIFICATION: *Claroideoglomus etunicatum (*W.N. BECKER & GERD.) C.WALKER & A.SCHÜßLER, 2010.	

Figure 2. Morphological and structural characteristics of the morphospecies with characteristics similar to *Glomus etunicatum* Becker & Gerdermann, 1977.

CONCLUSION

One of the most predominant morphospecies found in the salinity condition of soils of cattle farms sown with bentgrass was *Glomus etunicatum* Becker & Gerdermann, 1977, was reclassified as *Claroideoglomus etunicatum*. Although there is evidence that symbiosis with arbuscular mycorrhizal fungi affects and regulates several of the mechanisms involved in the salinity tolerance of bentgrass, many of the physiological aspects and the molecular basis of this regulation are unknown. What is amply demonstrated is the ecological importance of arbuscular mycorrhizal association for plant survival and growth under salt stress conditions, and hence its importance for agriculture under this condition.

ACKNOWLEDGEMENTS

The authors would like to thank the laboratory of microbiological research attached to the Faculty of Agricultural Sciences of the University of Sucre for carrying out this study.

AUTHOR CONTRIBUTION. Alexander Perez Cordero: experiment execution, data analysis. Donicer Montes V and Yelitza Aguas M, conceptualization, writing - revision and editing. All authors have read and approved the manuscript.

CONFLICT OF INTEREST. All the authors of the manuscript declare that they have no conflict of interest.

REFERENCES

1. Abdel Latef, A.A.H., Abu Alhmad, M.F., Kordrostami, M., Abo-Baker, A.-B.A.-E., Zakir, A., 2020. Inoculation with azospirillum lipoferum or Azotobacter chroococcum reinforces maize growth by improving physiological activities under saline conditions. J. Plant Growth Regul. 39, 1293–1306.
2. Al-Karaki, G. N. 2000. ‘‘Growth of mycorrhizal tomato and mineral acquisition under salt stress’’. *Mycorrhiza*, 10(2): 51-54, ISSN 0940-6360, 1432-1890, DOI 10.1007/s005720000055.
3. Al-Karaki, G. N. y Clark, R. B. 1998 ‘‘Growth, mineral acquisition, and water use by mycorrhizal wheat grown under water stress’’. *Journal of Plant Nutrition*, 21(2):263-276, ISSN 0190-4167, DOI 10.1080/01904169809365401.
4. Al-Karaki, G. N.; Hammad, R. y Rusan, M. 2001. ‘‘Response of two tomato cultivars differing in salt tolerance to inoculation with mycorrhizal fungi under salt stress’’. *Mycorrhiza*, 11(1): 43-47, ISSN 0940-6360, 1432-1890, DOI 10.1007/s005720100098.
5. Aliasgharzadeh, N.; Rastin, S. N.; Towfighi, H. y Alizadeh, A. 2014. ‘‘Occurrence of arbuscular mycorrhizal fungi in saline soils of the Tabriz Plain of Iran in relation to some physical and chemical properties of soil’’. *Mycorrhiza*, vol. 11(3):119-122, ISSN 0940-6360, 1432-1890, DOI 10.1007/s005720100113.
6. Azcón, A. C. y Barea, J. M. 1997. ‘‘Applying mycorrhiza biotechnology to horticulture: significance and potentials’’. *Scientia Horticulturae*, 68(1–4, 3. 1-24), ISSN 0304-4238, DOI 10.1016/S0304-4238(96)00954-5.
7. Azarbad, H., Niklinska, M., Laskowski, R., van Straalen, N.M., van Gestel, C.A.M., Zhou, J., He, Z., Wen, C., Roeling, W.F.M., 2015. Microbial community composition and functions are resilient to metal pollution along two forest soil gradients. FEMS Microbiol. Ecol. 91, 1.
8. Azadi, N., Raiesi, F., 2021. Sugarcane bagasse biochar modulates metal and salinity stresses on microbial functions and enzyme activities in saline co-contaminated soils. Appl. Soil Ecol. 167, 104043.
9. Barea, J. et al. 1999. Importancia de las micorrizas en el establecimiento y protección de las plantas en suelos degradados, medio ambiente, transferencias de tecnologías. Phytoma. España. 111: 56.
10. Baihui Hao, Zhechao Zhang, Zhihua Bao, Lijun Hao, Fengwei Diao, Frank Yonghong Li, Wei Guo. 2022. *Claroideoglomus etunicatum* affects the structural and functional

genes of the rhizosphere microbial community to help maize resist cd and la stresses. Environmental Pollution. 307:119559. https://www.sciencedirect.com/science/article/abs/pii/S0269749122007734

11. Giri, B. y Mukerji, K. G. 2003. ‘‘Mycorrhizal inoculant alleviates salt stress in *Sesbania aegyptiaca* and *Sesbania grandiflora* under field conditions: evidence for reduced sodium and improved magnesium uptake’’. *Mycorrhiza*, 14(5): 307-312, ISSN 0940-6360, 1432-1890, DOI 10.1007/s00572-003-0274-1.
12. Hidri, R., Metoui-Ben Mahmoud, O., Debez, A., Abdelly, C., Barea, J.M., Azcon, R., 2019. Modulation of C:N:P stoichiometry is involved in the effectiveness of a PGPR and AM fungus in increasing salt stress tolerance of sulla carnosa tunisian provenances. Appl. Soil Ecol. 143, 161–172.
13. International Culture Collection of Arbuscular & Vesicular- Arbuscular Mycorrhizal Fungi. (INVAM, by Morton J B., Curator, Good for Basic Research), 2019. URL disponible en: http://infotree.library.ohiou.edu/scripts/ redirect.html?id=1661.
14. Ma, Y., Rajkumar, M., Oliveira, R.S., Zhang, C., Freitas, H., 2019b. Potential of plant beneficial bacteria and arbuscular mycorrhizal fungi in phytoremediation of metalcontaminated saline soils. J. Hazard. Mater. 379, 120813.
15. Pérez CA, Vertel MM. 2010. Evaluación de la colonización de micorrizas arbusculares en pasto Bothriochloa pertusa (L) A. Camus. Rev MVZ Córdoba; 15(3):2165-2174.
16. Pérez C, Alexander; Peroza C, Víctor. 2013. Micorrizas arbusculares asociadas al pasto angletón (Dichathium aristatum Benth) en fincas ganaderas del municipio de Tolú, Sucre-Colombia Revista MVZ Córdoba, 18(1):3362-3369. https://www.redalyc.org/pdf/693/69325829016.pdf.
17. Peroza CV, Pérez CA. Efecto de parámetros físicos, químico y salinidad sobre la densidad poblacional y la colonización de micorrizas arbusculares en pasto Angleton en el municipio de Tolú, Sucre, Colombia. Rev Colombiana Cienc Anim 2010; 2(2):310-324.
18. Porras, S. A.; Soriano, M. M. L.; Porras, P. A. y Azcón, R. 2009. ‘‘Arbuscular mycorrhizal fungi increased growth, nutrient uptake and tolerance to salinity in olive trees under nursery conditions’’. *Journal of Plant Physiology*, 166(13): 1350-1359, ISSN 0176-1617, DOI 10.1016/j.jplph.2009.02.010.
19. Riaz, M., Kamran, M., Fang, Y., Wang, Q., Cao, H., Yang, G., Deng, L., Wang, Y., Zhou, Y., Anastopoulos, I., Wang, X., 2021. Arbuscular mycorrhizal fungi-induced mitigation of heavy metal phytotoxicity in metal contaminated soils: a critical review. J. Hazard. Mater. 402, 123919.
20. Rosendahl, C. N. y Rosendahl, S. 1991. ‘‘Influence of vesicular-arbuscular mycorrhizal fungi (*Glomus* SPP.) on the response of cucumber (*Cucumis sativus* L.) to salt stress’’. *Environmental and Experimental Botany*, 31(3): 313-318, ISSN 0098-8472, DOI 10.1016/0098-8472(91)90055-S.
21. Xu, Y., Ge, Y., Lou, Y., Meng, J., Shi, L., Xia, F., 2021. Assembly strategies of the wheat root-associated microbiome in soils contaminated with phenanthrene and copper. J. Hazard. Mater. 412, 125340.
22. Zhang, F., Liu, M., Li, Y., Che, Y., Xiao, Y., 2018. Effects of arbuscular mycorrhizal fungi, biochar and cadmium on the yield and element uptake of Medicago sativa. Sci. Total Environ. 655, 1150–1158.