**Cultivation Bufell grass (*Cenchrus ciliaris* L.) in symbiosis with arbuscular mycorrhizae produced mitigation of drought stress.**

Abstract

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| --- |
| ***Objectives****: The main objective was to study the symbiosis between Cenchrus ciliaris and Rizhophagus intraradices under water stress****. Study design****: The plant-mycorrhizal symbiosis provides hydraulic capacity to the plant, allowing it to be more efficient in arid soils. It also contributes to plant nutrition and improves soils for the recolonization of native species. Plants (P) were grown in a growth chamber with a 16:8 h light/dark cycle and a temperature of 25°C. Half of the plants were inoculated with mycorrhizae (AM), while the rest were not (NM). The treatments were 100%, 60%, and 40% water (drought levels).* ***Study location and duration:*** *The study was conducted in the Plant Physiology Laboratory, in the Ecology Area of the Department of Biology, Faculty of Chemistry, Biochemistry, and Pharmacy, National University of San Luis, from March to November 2024.* ***Methodology:*** *Morphological parameters were measured: length (L), fresh weight (FW), and dry weight (DW) of the aerial parts (A) and roots (R); physiological and biochemical parameters: photosynthetic pigments, proline, and malondialdehyde (MDA), were also measured.* ***Results:*** *No differences in mycorrhization were found between dyed roots of control, 60%, and 40% drought. Morphophysiological parameters were higher in AM plants compared to NM plants. Proline increased at 40% drought in NM plants, while it remained stable in AM plants. MDA decreased significantly in AM plants compared to NM plants, in all drought and control treatments.* ***Conclusion:*** *Mycorrhization conferred protection against stress. Mycorrhizae mitigate water stress in Buffel forage plants (C. ciliaris L.), a species recommended for semi-arid and arid environments in Argentina.* |

*Keywords: Arbuscular mycorrhizae Cenchrus ciliaris*, *Rizhophagus intraradices,* *water stress*

1. Introduction

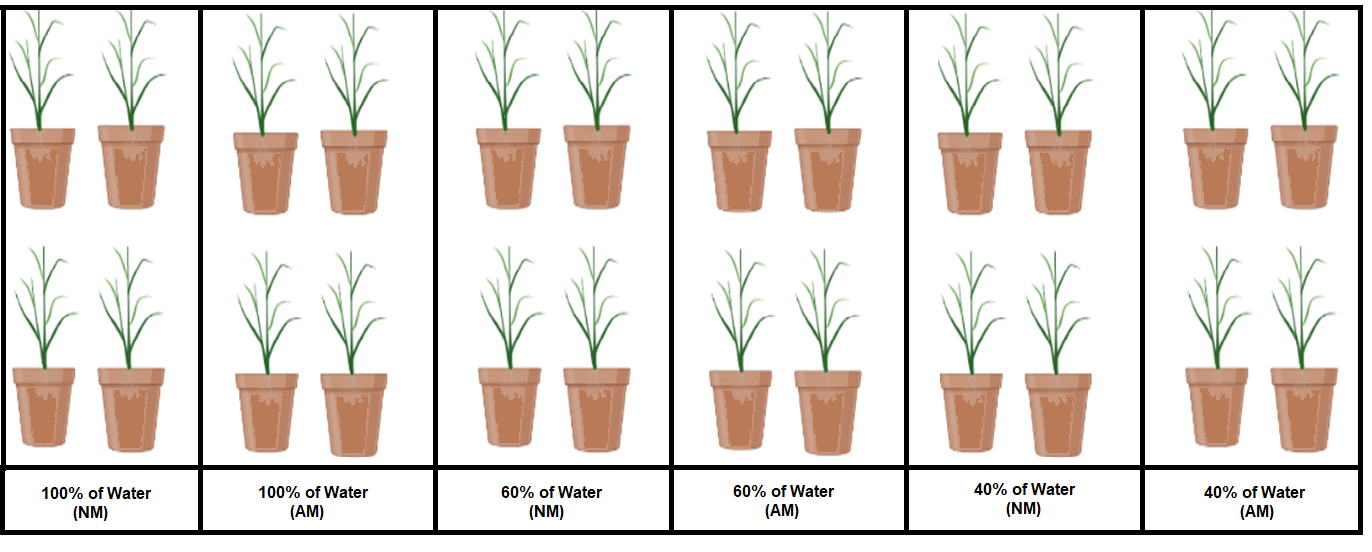
Soil is a dynamic biogeochemical natural resource that supports all the components comprising terrestrial ecosystems. Soil is known as the pedosphere, and the processes occurring within it are linked to ecosystem services and central to the biogeochemical cycles of nutrients and carbon that sustain life (Taboada, 2018). One of the characteristics of arid and semi-arid climate zones is the low availability of water, quantified based on average annual precipitation in relation to evaporation from a given geographic area. The trend toward desertification of the earth and soil degradation is mainly due to population growth, high pressure on the use of natural resources, poor agricultural practices, and human activity, which have contributed to climate change, the main effect of which is the increase in average temperatures of the planet, the decrease in fresh water reserves and the salinization of a high percentage of arable soil (Mazuela-Águila *et al.,* 2013; Ortiz-Bobea, *et al.*, 2021).Planting native and introduced grasses represents a productive option for improving rangeland conditions in arid and semi-arid zones, especially in areas where the best native forages have disappeared. Factors limiting the establishment of these grasses are low rainfall and low soil fertility (Loredo *et al.,* 2004; Lejia Loredio et al., 2016) . The use of mulch helps prevent soil erosion, provides plant-available nutrients, increases microbial activity that transforms organic matter, and retains soil moisture (Márquez *et al.,* 2003). (Canchani, *et al*., 2018). Buffelgrass (*Cenchrus ciliaris* L.) belongs to the *Poaceae* family, subfamily *Panicoideae* and tribe *Paniceae*. It is a vigorous, summer-growing, perennial grass that is tolerant of drought and high temperatures (45°C), and some cultivars are cold-tolerant (-10°C) (Ayerza *et al.,* 1981). It is a species native to Africa that begins to sprout with spring rains and dries out with the first frosts. The optimal growth temperature is 25-35°C; germination 25°C and 300 mm of rain during its growing season. Arbuscular mycorrhizae (AM) are present in existing agricultural systems and are the most widespread type of symbiosis in nature, capable of establishing associations in 80–85% of vascular plants (Castillo-González, 2009). Arbuscular mycorrhizal fungi (AMF) are obligate symbionts, which mean they cannot complete their biological cycle in the absence of the host plant and must remain associated with the plant root to obtain carbohydrates from photosynthesis. In return, the fungus supplies the plant with mineral nutrients and water extracted from the soil, extending the plant roots beyond the nutrient-depleted zone created around them (Ruiz Lozano *et al.,* 2001; Cavagnaro, 2014). AMF have a significant influence on plant physiology and water relations under stress conditions. In arid and semi-arid ecosystems, mycorrhization increases water acquisition in various plant species, decreasing lipid peroxidation, increasing K+: Na+ and Ca2+: Na+ ratios and increasing the production of glycine, betaine and proline (Harris-Valle *et al*., 2009). AMF contribute to improving the physical and chemical properties of the soil by enriching organic matter and stimulating the formation of particle aggregates that improve soil structure and stability, reducing erosion and increasing its water retention capacity (Finlay, 2008). In areas degraded by land-use change, AMs play an important role in ecological recovery (Carrillo-Saucedo *et al.,* 2022). AMF play a key role in the establishment and development of most plants, improving productivity, survival and resistance to pathological factors; improving the productive capacity of soils such as those affected by desertification, salinization, water and wind erosion (Castillo González, 2009) and can partially or totally reduce the use of fertilizers (Cruz-Hernández *et al.,* 2014; Albarado y Castillo,2018). Symbiosis favors water absorption and allows for improved plant development in conditions of drought, salinity, and cold, particularly in forage species from semi-desert environments (Pedranzani *et al.,* 2015 a and b). Khan *et al.* (2008) observed that the association of AM with *C. ciliaris* L. in two water regimes (100 and 50% field capacity) increased water use efficiency in both cases. Díaz Franco y Garza-Cano(2006) observed that mycorrhizal root colonization was 42% in *C. ciliaris* and that there were significant increases in chlorophyll content, leaf protein, and dry and root biomass. Our objective was to study the effect of the symbiotic relationship of *C. ciliaris* L. with AM in response to drought, through morphological, physiological and biochemical parameters

2. Material and Methods

**2.1- Sowing and growing plants.**

*C. ciliaris* L. It is a megathermic pasture, of South African origin, which adapts very well to arid and semi-arid environments (Ayerza, 1981). Scarified seeds were germinated in Petri dishes on moist absorbent paper in an oven at 30 C° and darkness. The seedlings were sown one per pot in a soil: perlite mixture (1:1) sterilized at 70 C° for 96 hs. The experimental design was 24 pots of 250 ml, 50% were inoculated with 1 cm3 of commercial mycorrhiza (*Rhizophagus intraradices* spores) and the rest were not. Non-mycorrhizal (NM) and mycorrhizal (AM) plants grew in a sowing chamber at 24-26 ºC; with a photoperiod of 16:8, light:dark and were watered weekly until they reached a height of 25 cm and from there the stress treatments were started.

Treatments were carried out weekly for one month: Control = 100 ml of water (field capacity); 60% = 60 ml of water (60% field capacity); 40%= 40 ml of water (60% field capacity). The plants were harvested, and LA (shoot length) and AFW (aerial fresh weight) were measured. The roots were washed, and RL (root length) and RFW (root fresh weight) were measured. They were placed in a drying oven at 70°C for 72 hours, and ADW (shoot dry weight) and RDW (root dry weight) were measured. Each treatment had 4 replicates as indicated in Figure 1



**Figure 1:** Experimental design

**2.2-Quantification of mycorrhizal infection in roots** The Phillips and Hayman (1970) differential staining method was used, which consisted of rinsing the roots and staining them with a specific dye for the fungal chitin wall. To quantify root colonization, the grid line intersection method was used (Giovannetti and Mosse, 1980). The percentage of mycorrhized roots (% MIC) was obtained from the formula: % MIC = (100 m /T), where "m" is the number of "mycorrhized" intersections and "T" is the total number of intersections observed.

**2.3- Chlorophyll a, b, and carotenoids determination.** 100 mg of aerial foliage tissue was collected and homogenized in a mortar with 80% acetone. It was then filtered through a funnel filled with filter paper and measured using a spectrophotometer. To quantify chlorophyll a and b, absorbance was measured at 646.6 nm and 663.6 nm, respectively, and carotenoids at 470 nm. 80% (v/v) acetone was used as a blank. Samples were taken in triplicate. Endogenous chlorophyll content was calculated according to Porra (2002) and carotenoids content according to Sims and Gamon (2002). Results are expressed as mg/g DW (for chlorophylls) and mg/PF (for carotenoids).

**2.4-Proline Determination** Proline determination was performed according to the method of Bates et al. (1973). Absorbance was read at 520 nm using the following blanks: 2 ml of distilled water + 2 ml of acid nitrohydrin + 2 ml of glacial acetic acid. Proline concentration was determined from a standard curve and is expressed as proline content per unit of fresh weight

**2.5- Malondialdehyde (MDA) determination.** 200 mg of leaves was homogenized with liquid nitrogen and extracted with 600 ul of extraction buffer (50 mM Tris-Cl pH 7.5 + 0.1 mM EDTA + 2% Triton) shaken, and centrifuged at 10,000 rpm for 30 minutes at 4°C. Then 200 ul of supernatant from each sample plus 1 ml of MR (0.375% TBA and 15% TCA) were added; the mixture was shaken and placed in a hot water bath at 95-100°C for 15 min and on ice for 5 min. The mixture was centrifuged for 5sec. at 2000 rpm and read in a spectrophotometer at 535 nm wavelength. For the standard curve, hydrolyzed TMP was prepared at a concentration of 1:100 (Morales and Munné-Bosch, 2024)

**2.6- Statistical Analyses** Statistical analyses were performed with the GraphPad Prism Version 8.0.2 (263). The morphological parameters were analyzed by Student T parametric and percentage of colonization, pigments, proline and MDA results were analyzed by multifactorial ANOVA. Significant differences among treatments were identified using the Tukey B test (p < 0.05). (Hood and Wilson, 2001)

3. Results

**3.1- Mycorrhizal Quantification**

In the quantification of mycorrhizae using the method (Giovannetti and Mosse, 1980), it was observed that there were no significant differences between the percentages of mycorrhization among the different treatments (Table 1) 60%, 57,1%, 44,6%

**Table 1: Percentage of mycorrhization of *Cenchrus ciliaris* .**

|  |  |  |  |
| --- | --- | --- | --- |
| **Treatments** | **Roots accountability** | | **Percentage of mycorrhization** |
| 100% Water  60% water | 310  268 | 60,0 % a  57,1 %a | |
| 40% of water | 204 | 44,6 %a | |
|  |  |  | |

*Different letters mean significant differences, \* P < 0.05 significant from normal control*

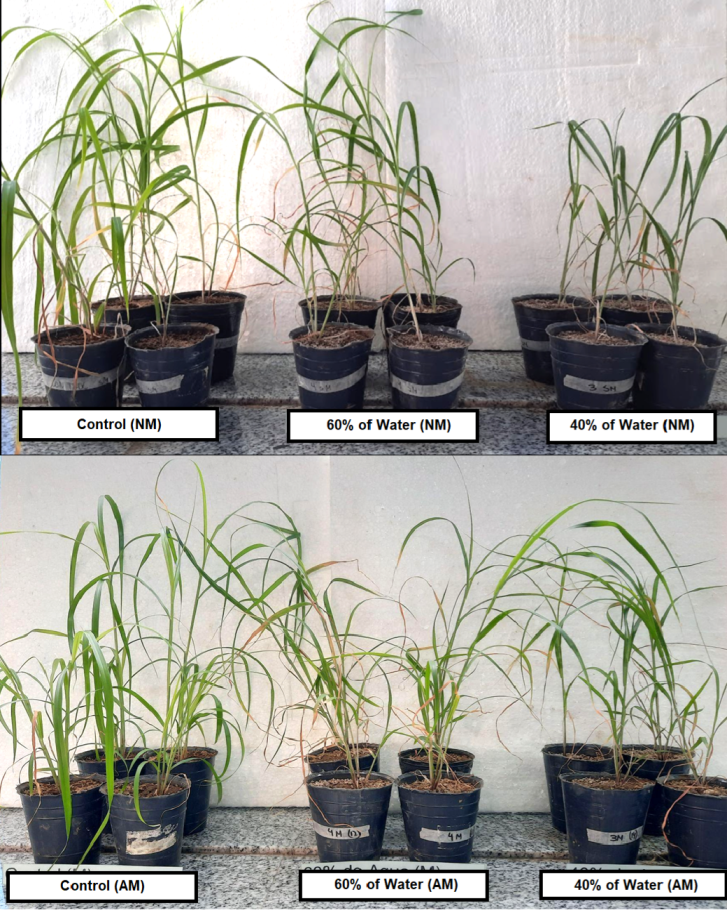
**3.2- Morphological and growth parameters.**

In the control treatment, all measured parameters were significantly higher in AM plants than in NM plants. In the water-restricted treatment (60% of water), all parameters increased, except for ADW. In the 40% water treatment, AM plants also showed greater increases than NM plants, except for AFW and RFW (Table 2 and Figure 2)

**Table 2: Morphological parameters of *Cenchrus ciliaris* L in Mycorrhizal (AM) and Non-mycorrhizal (NM) plants (AL) aerial large, (RL): root large; (AFW) aerial fresh weight, (RFW) root fresh weigth, (ADW) aerial dry weight (RDW) root dry weigth.**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Parameters** |  | **Control** | | | **60% of water** |  | | **40% of water** |  |
| **AL (cm)**  **RL (cm)**  **AFW (g)**  **RFW (g)**  **ADW (g)**  **RDW(g)** | NM  60 b  15f  6b  1,5d  1,1d  0,4f | | AM  **69a**  **19e**  **9a**  **2,9c**  **2c**  **0.8e** | NM  52c  15f  4c  1e  1,4d  0,5f | | AM  **59a**  **21e**  **4,9b**  **1,6d**  1,1d  **0,8e** | NM  45d  15f  2,8c  0,5e  1e  0,4f | | AM  **52c**  **28e**  2c  0,5e  **2,2c**  **0,6e** |
|  |  | |  |  | |  |  | |  |

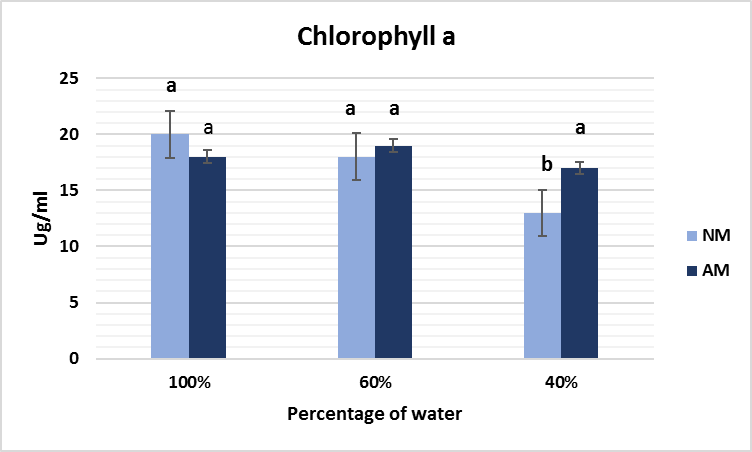
*Different letters mean significant differences, \* P < 0.05 significant from normal control*

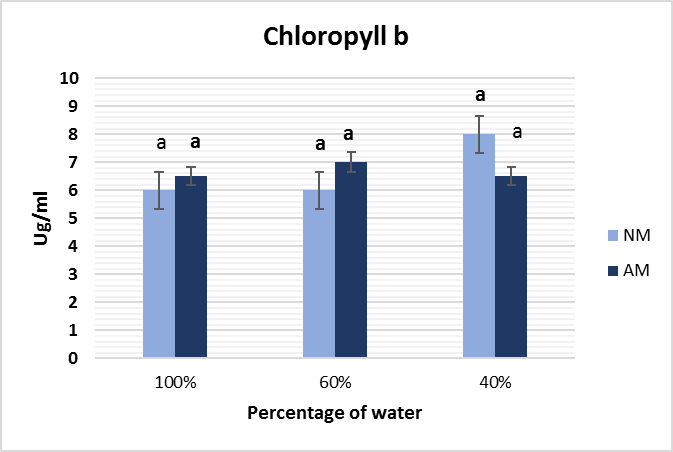


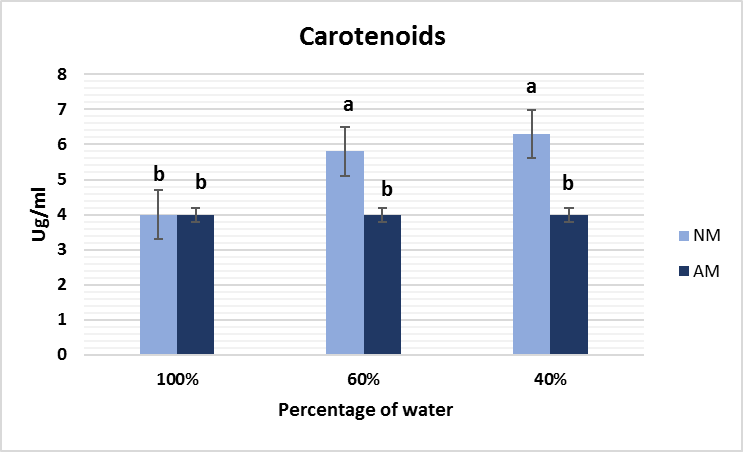
**Figure 2: Images of *C. ciliaris* L. (*Buffel grass)* plants under control and drought treatments**

**3.3- Photosynthetic pigments.**

Photosynthetic pigments indicate the physiological state of the plant under water stress. Figure 3A shows that under control conditions and with 60% water, chlorophyll a did not change in NM and AM plants. With 40% water, AM plants increased their chlorophyll a content (Figure 3 A). Chlorophyll b did not change in any of the treatments, in AM and NM plants (Figure 3 B). Carotenoids are protective pigments that increase in the presence of stress to protect the photosynthetic apparatus. In our experiment, carotenoids increased under stress conditions (60 and 40% water) in NM plants. In AM plants (100, 60 and 40%), there were no significant differences due to mycorrhizal protection (Figure 3 C)

**A**

**B**

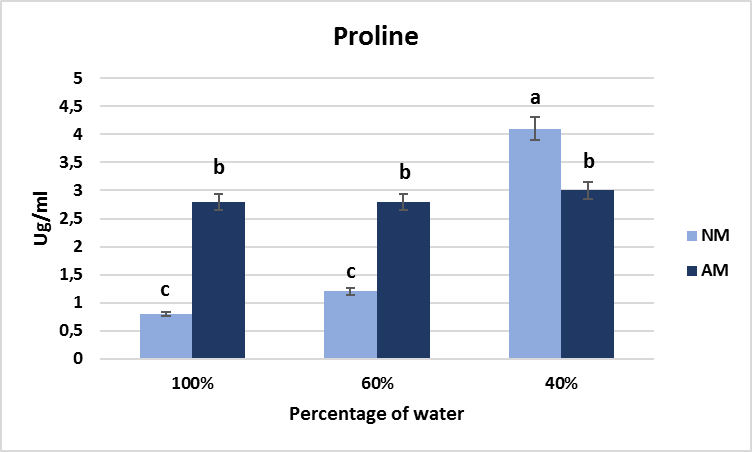
**C**

**Figure 3: Photosynthetic pigments of *C. ciliaris* under water stress. A: Chlorophyll a; B: Chlorophyll b; C: Carotenoids** in Non-mycorrhizal (NM) and Mycorrhizal plants (AM**).** *Different letters mean significant differences, \* P < 0.05 significant from normal control*

*Mean ± S.E.M = Mean values ± Standard error of means of four experiments*

**3.4- Proline**

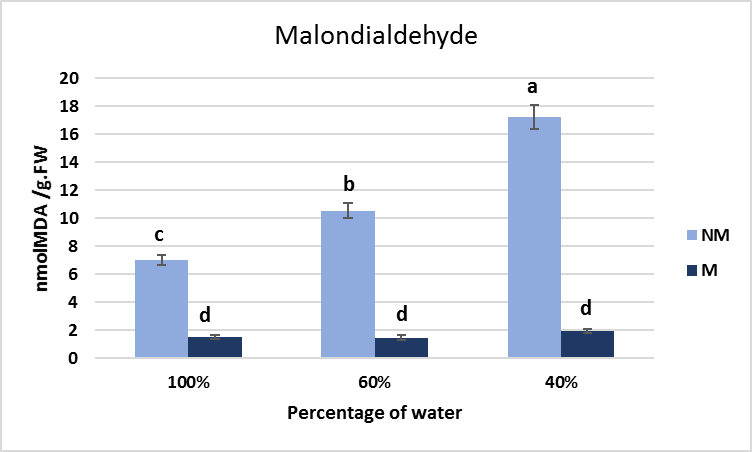
The function of proline, like any compatible osmolyte, is to increase cellular water retention under abiotic stress. In NM plants, proline increased significantly with 40% water. In AM plants, proline remained unchanged at all water stress levels, likely due to the protection of mycorrhizae (Figure 4).



**Figure 4: Proline of *C. ciliaris* under water stress.) in Non-mycorrhizal (NM) and Mycorrhizal plants (AM).** *Different letters mean significant differences, \* P < 0.05 significant from normal control Mean ± S.E.M = Mean values ± Standard error of means of four experiments*

**3.5- Malondialdehyde**

Reactive oxygen species (ROS) increase under abiotic stress. These are potentially toxic molecules that cause nonspecific oxidation of membrane proteins and lipids and are linked to DNA damage. This results in an increase in malondialdehyde (MDA) (Murube Torcida,2014). In our study, water stress caused a significant increase in MDA as stress increased in NM plants. In both control and water-stressed AM plants, there was no synthesis of this compound, demonstrating mycorrhizal protection (Figure 5)



**Figure 5: Malondialdehyde content of *C. ciliaris* under water and salt stress. .NM: Non mycorrhizal plants. AM Mycorhyzal plants.** *Different letters mean significant differences, \* P < 0.05 significant from normal control Mean ± S.E.M = Mean values ± Standard error of means of four experiments*

**Discussion**

*Growth*

Morphophysiological parameters (AL, RL, AFW, RFW, ADW, RDW) were higher in control AM plants. Under 60% water stress, all parameters were higher in AM plants except ADW. Under 40% water stress, AM plants performed the same except for AFW and RFW. NM plants behaved similarly to Tommasino (2018). For Glycine max, plant height and weight were lower in NM plants (Porcel et al., 2006) and alfalfa (Achiary et al., 2024). Mycorrhizae contributed to biomass production in control and stressed plants. Mycorrhizae contribute to resource acquisition, adaptation, and tolerance to water stress.

*Percentage of mycorrhization in roots.*

The mycorrhization percentage was around 50% in all treatments in *C. ciliaris* for two months, meaning that stress did not affect mycorrhization. Díaz Franco and Garza-Cano (2006) found a mycorrhizal colonization rate of 42% in the same species after seven months, using soil and sorghum roots inoculum, in our study, commercial spore powders were used in the substrate. In different species of the genus *Prosopis* subjected to salinity, mycorrhizae concentrations of up to 80% were found (Scambato et al., 2013). In *Medicago sativa*, under water and salt stress, mycorrhization percentages were close to 60% and similar to the control (Pedranzani et al., 2021).

*Photosynthetic Pigments*

Chlorophyll a decreased in C. ciliaris L. under water stress in NM plants, while remaining constant in AM plants. Chlorophyll b did not differ at any stress level, and carotenoids increased as a protective factor at both water stress levels in NM plants, while remaining constant in AM plants. Chlorophyll tends to decrease more rapidly than carotenoids under stress or senescence (Cervantes-Sanchéz, 2014). Similar results were found in Zea mays L. (Samano Leiva, 2014). The decrease in chlorophyll a is due to the inability to efficiently capture radiant energy, and the increase in carotenoids is related to the detoxification of reactive oxygen forms formed during photosynthesis due to stress. Similarly, the decrease in chlorophylls with respect to carotenoids may be a trait of resistance to drought, so they can be classified as tolerant to this type of stress (Argentel et al., 2006).

*Proline.*

Proline is an osmoprotectant linked to plant tolerance to drought and salinity. Its function is to maintain tissue turgor, protecting the plant from desiccation. It is also linked to nitrogen storage for rehydration and non-enzymatic antioxidant defense (Ferres Jaunsolo and Monsa, 2008). Proline in C. ciliaris L. AM under water stress did not vary at any of the stress levels (60% and 40% water) compared to the control. In contrast, in NM plants, proline content increased significantly at both water stress levels. Similar results were found for Pistacia vera L. (Abbaspour et al., 2012), while the results are contrasting for Medicago sativa (Pedranzani et al., 2021) and Zea mays (Samano, 2014). Therefore, *C.ciliaris* NM plants suffered stress more severely, while in AM plants, mycorrhizae mitigated the lack of water and helped retain soil moisture.

*Malondialdehyde*

The most common consequence of most abiotic stresses is an increase in reactive oxygen species (ROS). This leads to an increase in malondialdehyde (MDA) (Murube *et al.,* 2014). During water stress, an increase in MDA was observed in NM plants as stress increased, while AM plants did not change their MDA content. In *Leymus chinensis* (Zhen *et al.,* 2007) and wheat (Amin *et al.,* 2023), MDA increased with increasing water stress in both AM and NM plants. In tomato plants, the results were similar to ours; mycorrhizal plants under water stress had lower MDA contents than NM plants (Ruscitti *et al.,* 2015). It can be seen that mycorrhizae mitigate water stress in *C. ciliaris* L.

4. Conclusion

*Cenchrus ciliaris* l is a plant adapted to poor and dry soils of south Africa. it has been introduced in argentina with the purpose of planting it in marginal areas, where water is scarce and soils are poor. there have been good previous agronomic results with this species, but the interesting thing about this article is that it combines a forage species with commercial arbuscular mycorrhizae (*Rizhofagus intraradices*) achieving greater performance. greater growth, greater vigor in the leaves, greater root development has been achieved, the photosynthetic rate, that is, the production of biomass remained constant in symbiosis. carotenoids (protective pigments) remained stable unlike plants without symbiosis, which increased as a form of defense, such as proline and mda. therefore, we conclude that cechrus ciliaris together with commercial mycorrhizae is a good set against desertification, poor soils and lack of water in the context of climate change.

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**Data Availability Statement:** The data that support the findings of this study are available from the

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**Conflicts of Interest:** The authors declare no conflict of interest.

Disclaimer (Artificial Intelligence) Option 1:

Author(s) hereby declare that no generative ai technologies such as large language models

(chatgpt, copilot, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

Ethical issues

This work respects the ethical standards of non-discrimination, non-pollution, and a healthy work environment.

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