

Functional Diversity of Mycorrhizal Fungi Has Differential Effects on Salinity Tolerance of *Acacia seyal* (Del.) Seedlings

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Abstract

Acacia seyal is a leguminous plant that plays an important role in the ecosystem of Sahelian zone by producing gum, wood and fodder. The growth of *A. seyal* is subject to many constraints as salinity which can affect the development of this tree. Therefore, soil microorganisms can help *A. seyal* to better tolerate the effects of negative environmental stresses. The contribution of arbuscular mycorrhizal fungi (AMF) to the salt tolerance of *A. seyal*, was evaluated by testing the effects of eight different arbuscular mycorrhizal fungi (AMF) isolates in the performance of *A. seyal* seedlings subjected to different levels of salinity (0, 340 and 680 mM). The results based on growth parameters of shoot and root parts, shoot mineral N, P, K and Na content as well as survival rates and mycorrhization showed that AMF improved mineral nutrition of *A. seyal* seedlings during salt stress. The combination between AMF and salinity provided evidence that the efficiency of AMF isolates were variable in improving mineral nutrition and mortality rate for *A. seyal* seedlings related to the level of salt stress. However, the effects of inoculation were variable depending to the AMF isolate associated with seedlings and the level of salinity, suggesting that interactions between plants and AMF can be modulated by both AMF diversity and the type and level of abiotic factors. *Rhizoglyphus intraradices* was more efficient at 680 mM NaCl in plant growth and mineral uptake while *Glomus deserticola* did not promote a better plant development than most of the other species inoculated to seedlings.

Keywords

Arbuscular Fungi, Salt Stress, Symbiotic Efficiency, *Acacia seyal*, Leguminous Tree, Functional Diversity

1. Introduction

Acacia seyal (Delile) is one of over 60 leguminous African acacia plant species (family Fabaceae, subfamily Mimosoideae) growing throughout many countries from Senegal to Sudan, and in the eastern and southern areas of Africa [1]. Adapted to a semi-arid environment, *A. seyal* is an excellent nitrogen-fixing species that grows in many soil types including periodically flooded [2] and moderately saline soils. A multipurpose tree, the wood of *A. seyal* is particularly cherished for fire wood and charcoal production, and branches are used for fencing. A gum (gum talha) is also collected from the tree [3]. Leaves and shoots provide precious forage, as do the fruit, and branches are often lopped by herders when herbaceous forage diminishes in the dry season. *A. seyal* also plays a major role in fuel and fodder production in countries at the southern edge of the Sahara Desert [2].

Like most plants in semi-arid ecosystems, *A. seyal* is subject to various abiotic constraints including salinity, which remains a major problem for the fertility of soils, particularly in the Sahelian zone, affecting the growth and productivity of poorly adapted plant species. The survival and growth of *A. seyal* in such hostile environments could be partly due to its association with beneficial soil microorganisms including arbuscular mycorrhizal fungi (AMF), which are obligate symbionts forming associations with a large number of terrestrial plant species [4] [5]. The AMF form hyphal networks through the soil and forage efficiently for nutrients (primarily P, but also Zn, N, and other nutrients) that are delivered to their host plants in exchange for carbon [4] [6] [7] [8]. This can also improve soil structure via enmeshment and entanglement mechanisms and the exudation of polysaccharides that help to bind soil particles together [9].

The symbiotic association between plants and AMF is strongly affected by environmental and edaphic conditions [10] [11] including salinity [12]. This can restrain AMF spore germination, colonization capacity, and hyphal development [13]. However, many researchers have reported that AMF can enhance the ability of plants to cope with salinity [14]-[20], leading to improved growth and productivity under saline conditions [21] [22] [15] [17]. The benefits of symbiosis under saline conditions include increased nutrient uptake compared with plants without mycorrhizal associations, accumulation of compounds regulating osmotic adjustment in the roots, and increase in photosynthesis and water use efficiency [14] [23]. AMF symbiosis has been demonstrated to increase resistance to soil salinity in a variety of host plants [19] [24] [25] [26] [27]; however, the intimate mechanisms are not always well understood [18]. The effects of sa-

linity on fungal colonization capacity appear to depend on the host plant and fungal species in addition to the growing conditions [14]. Recent studies collectively indicate that there exists a functional diversity among AMF, as different combinations of host plant and AMF have different impacts on morphology, nutritional status, symbiotic efficiency, and gene expression patterns in the symbiosis [28] [29] [30] [31].

Despite the important role of AMF symbiotic associations in the Sahelian zones, the functional diversity of AMF species colonizing *A. seyal*, and their ability to influence tolerance to salt stress, is still unknown. In this study, we compared the efficiency of eight different AMF in their ability to promote the early-stage growth and subsequent survival of *A. seyal* and the acquisition of the mineral nutrients nitrogen, phosphorus, potassium, and sodium. We hypothesized that the potential benefits of the symbiosis vary with the AMF species in symbiosis with *A. seyal* and that the AMF taxa that help plants to tolerate salt stress do so by improving nutrient uptake. *A. seyal* is an important tree species for semi-arid ecosystem; this work will allow greater understanding of how the symbiosis between *A. seyal* and AMF works, and allow development of AMF inocula to improve plant production in saline soils.

2. Materials and Methods

2.1. Plant Material and Soil Substrate

Seeds of *A. seyal* were harvested at maturity from adult trees in Bambey (14°42'N, 16°28'W), located in the groundnut basin of Senegal and characterized by a large density of *A. seyal* trees. The seeds were sterilized and scarified in 95% sulfuric acid (H₂SO₄) for 30 min and then rinsed four times with distilled water before immersion in sterilized distilled water for 2 hours to facilitate germination. The seeds were germinated in Petri dishes containing agar (0.8%) and placed in the dark at 30°C. After 3 days, the pre-germinated seeds were planted in plastic bags containing 1.5 kg of Bambey soil at one seed per bag and transferred to the greenhouse of Laboratoire Commun de Microbiologie IRD/ISRA/UCAD, Bel-Air, Senegal. The soil substrate used was collected at a depth of approximately 0 - 20 cm from the *A. seyal* rhizosphere in Bambey. The sampled soil was sandy with 11.5 mg·kg⁻¹ available phosphorus, 0.238 mg·kg⁻¹ available nitrogen, 0.4 mg·kg⁻¹ available potassium, and pH 5.5. The soil substrate was sterilized twice at 120°C by autoclaving for 1 h each time before using to fill plastic bags (1.5 kg of soil per bag) for cultivation of *A. seyal* seedlings. Seedlings were watered regularly with distilled water.

2.2. Mycorrhizal Inoculum Preparation

The following fungal species used for inoculation were provided by Laboratoire Commun de Microbiologie IRD/ISRA/UCAD, Bel-Air, Senegal: *Rhizophagus intraradices*, *Funneliformis mosseae*, two isolate of *Glomus aggregatum* (IR. 27 and DAOM 227 128), *Rhizophagus manihotis*, *Rhizophagus fasciculatus*, *Funne-*

liformis verruculosum, and *Glomus deserticola* (isolated from Senegalese soil). Each mycorrhizal species was maintained in monoculture in a greenhouse with maize (*Zea mays* L.) seedlings as host plants, growing in a sterilized substrate [32] [33]. After a four-month period, spore cultures of AMF were extracted by the wet sieving method [34] to check the viability of the spores before inoculation of *A. seyal* seedlings.

2.3. Experimental Design

Three-day-old *A. seyal* seedlings in plastic bags were arranged in a completely randomized experimental design consisting of nine different AMF treatments (eight AMF species and control) and three different NaCl treatments (0, 340, and 680 mM NaCl). Six replicates were performed, resulting in a total of 162 seedlings. The duration of the experiment was 18 weeks and the following combined treatments were tested:

- Non-mycorrhized plant controls (C),
- plants inoculated with *Rhizophagus intraradices* (R. intra)
- plantes inoculées avec *Funneliformis mosseae* (F. moss)
- plants inoculated with *Glomus aggregatum* (IR. 27) (G.a 1)
- plants inoculated with *Rhizophagus manihotis* (R. mani)
- plants inoculated with *Rhizophagus fasciculatus* (R. fasc)
- plants inoculated with *Glomus aggregatum* (DAOM 227 128) (G.a 2)
- plants inoculated with *Funneliformis verruculosum* (F. verru)
- plants inoculated with *Glomus deserticola* (G. desert)

2.4. Seedling Inoculation and NaCl Application

For each *A. seyal* seedling, inoculation with AMF was carried out after transplanting seedlings in plastic bags by placing 20 g of inoculum (sterile substrate containing a mixture of around 120 spores, hyphae, and fine roots) of the species tested at a depth of 2 - 3 cm as close as possible to the vicinity of *A. seyal* roots. Non-inoculated seedlings received the same amount of sterilized substrate without AMF.

A single application of NaCl (100 ml solution at 340 and 680 mM per plant) was supplied to *A. seyal* seedlings after 3 weeks of growth with AMF in order to allow mycorrhization to take place. Control plants received 100 ml distilled water. Plants were maintained for a further 15 weeks in the greenhouse and watered regularly to field capacity without fertilization.

2.5. Shoot Growth Measurement and Mortality Rate Determination

Plant length measurements were performed once every 3 weeks until 18 weeks of the experiment. The first measurement was carried out 3 weeks after inoculation and before the application of NaCl.

The mortality rate for each treatment was determined as a percentage by using the following formula:

$$\frac{\text{total number of dead seedlings}}{\text{total number of plants}} \times 100$$

2.6. Shoot and Root Dry Weight Determination

For each treatment, six *A. seyal* seedlings were harvested after 18 weeks of cultivation, and the shoot parts were separated from the root parts before placing them at 70°C for 3 days to determine the dry weight.

2.7. AMF Root Mycorrhization

At the end of the experiment, AMF root colonization was analyzed using a random sampling of the root system, with three replicates for each treatment. Fine roots were sampled and thoroughly rinsed with tap water to remove sand particles and then stained by using the method of Phillips and Hayman [35]. The histological examination of root colonization was performed by microscopy of 10 root fragments of about 0.5 cm in length, placed between slides and coverslips with a few drops of glycerol, according to the method of Trouvelot *et al.* [36].

2.8. Shoot Chemical Analysis

The shoot parts of the dried plants were crushed and analyzed to determine the content of phosphorus, sodium, nitrogen, and potassium in relation to the mean weight of the dried plants.

Total nitrogen analysis was carried out by the Kjeldahl digestion method [37]. The total phosphorus content was determined by automatic colorimetry with formation of a yellow phosphomolybdate complex that is reduced by ascorbic acid, according the method of Murphy and Riley [38]. Exchangeable Na and K were extracted with ammonium acetate at pH 7 and measured by atomic absorption spectrometry.

2.9. Statistical Analysis

The treatments were performed in two factorial combinations (salinity and AMF) with 6 replicates. The experimental data were subjected to analysis of variance (ANOVA) to study the effect of AMF and salinity level on seedlings height, shoot and root dry matter, mineral content and mycorrhization. Means were compared by Student-Newman-Keuls multiple range test and the significance level was fixed at $p < 5\%$. All statistical analyses were performed with the software R 3.0.1 (R Core Team, 2013).

3. Results

3.1. Height of Plants

A. seyal plant height was dependent on the level of NaCl and the mycorrhizal species applied. All growth curves (**Figure 1**) generally showed the same trend with faster growth between the 3rd and 12th week and slower growth beyond the 12th week. In the absence of NaCl (0 mM), height was fairly homogeneous until

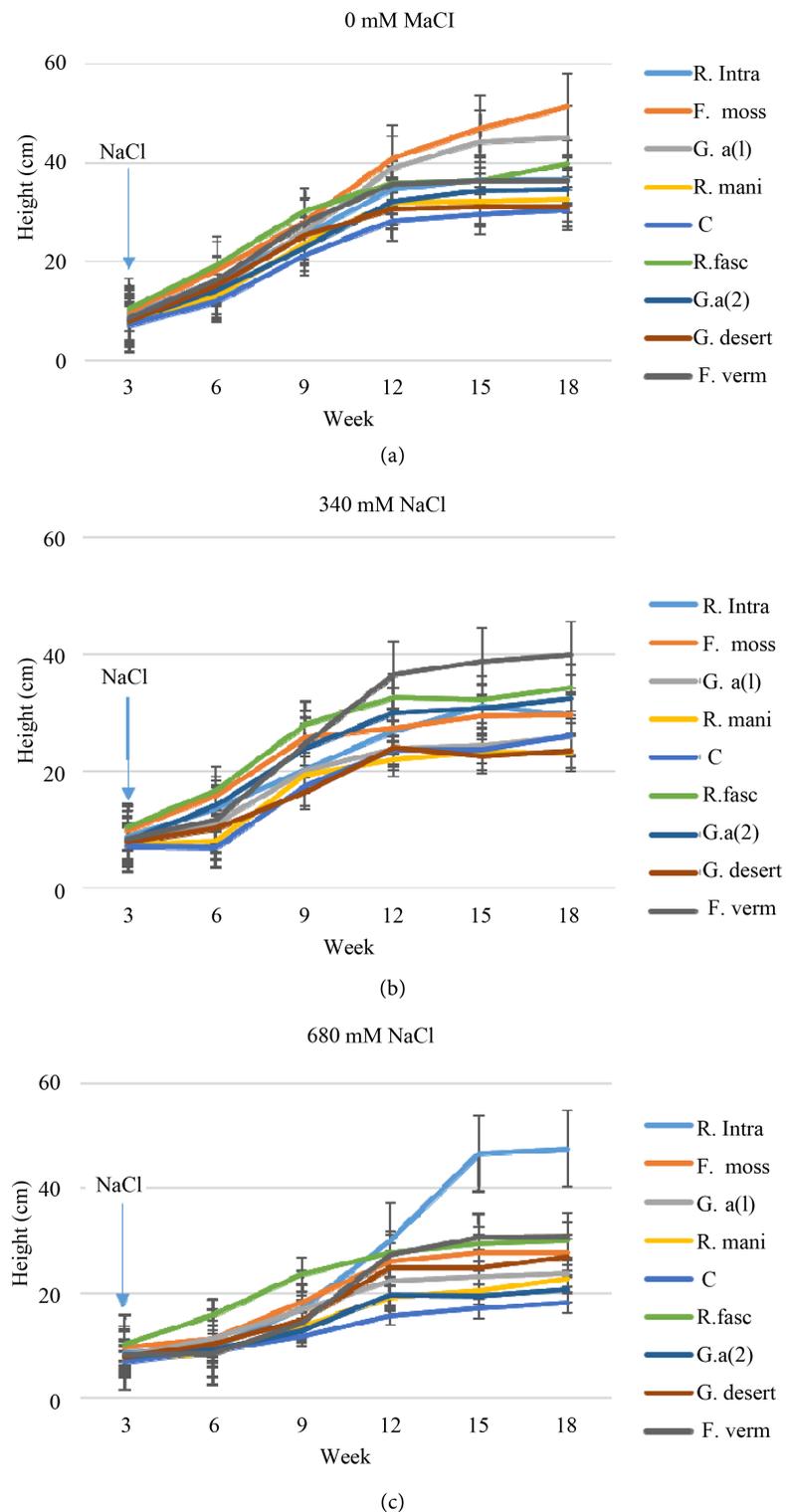


Figure 1. Height of *A. seyal* seedlings without NaCl (a) and with 340 (b) and 680 (c) mM of NaCl added after 3 weeks of growth. Seedlings inoculated with *R. intraradices* (R. intra), *F. mosseae* (F. moss.), *G. aggregatum* IR. 27 [G.a (1)], *G. aggregatum* DAOM 227 128 [G.a (2)], *R. manihotis* (R. mani), *R. fasciculatus* (R. fasc.), *G. deserticola* (G. desert), or *F. verruculosum* (F. verru), and the non-inoculated control (C). Vertical bars represent standard deviations of six replicates.

the 12th week regardless of the AMF species inoculated, with an average plant size of 30.30 cm (**Figure 1(a)**). Beyond this period, differences among plants inoculated with different AMF species were more obvious. Thus, the uninoculated controls were the shortest at the 18th week (30.32 cm), whereas plants inoculated with *F. mosseae* (51.32 cm) showed the greatest degree of growth, followed by plants inoculated with *G. aggregatum* IR. 27 (45.2 cm) and *R. fasciculatus* (39.75 cm). All other mycorrhizal treatments produced plants of similar height.

When 340 mM NaCl was applied to the growth substrate, seedling growth rates became more distinct from each other from the 6th to the 12th week (**Figure 1(b)**). After this period, the curves were divided into three groups according to the average size of the plants. Plants inoculated with *F. verruculosum* were the tallest, with a mean height of 40 cm at the 18th week. An intermediate group consisting of plants inoculated with *R. fasciculatus*, *G. aggregatum* DAOM 227 128, *F. mosseae* and *R. intraradices* had mean heights of, respectively, 34.30, 32.45, and 29.75 cm. The plants inoculated with the other AMF and the control (26.08 cm) were shorter.

With 680 mM NaCl, the differences in height between the plants became more obvious with time (**Figure 1(c)**). The plants inoculated with *R. fasciculatus* attained a mean height of 15.67 cm after 6 weeks of culture, which was greater than that of plants in all other treatments. From the 12th week, the plants inoculated with *R. intraradices* showed the greatest increase in height, with a mean height of 29.92 cm. At this level of salinity, uninoculated plants displayed less growth than all other plants, reaching a mean height of 18.16 cm at the 18th week of culture in comparison with 47.5 cm for plants inoculated with *R. intraradices*.

3.2. Shoot and Root Biomass Dry Weight

The biomass dry weight of the shoot parts of control plants decreased with increasing salt concentration (**Figure 2**). The shoot biomass dry weight of inoculated plants was generally higher than that of the control except for *R. manihotis* following treatment with 0 and 340 mM NaCl.

With 0 mM NaCl, plants inoculated with *R. intraradices* and *F. mosseae* had the greatest shoot biomass after 18 weeks with, respectively, 1.08 and 0.89 g. The plants inoculated with *R. manihotis* or *G. deserticola* and the controls showed the smallest shoot biomass dry weights with, respectively, 0.58, 0.66, and 0.67 g. For plants inoculated with *G. aggregatum* IR. 27, *R. fasciculatus*, *G. aggregatum* DAOM 227 128, and *G. deserticola*, the biomass of shoots parts declined with increase in salt concentration.

With 340 mM NaCl, the greatest dry weight of shoot parts was noted in plants inoculated with *F. verruculosum* (0.94 g). By contrast, plants inoculated with *R. manihotis* and the controls had lesser shoot dry matter weights with, respectively, 0.36 and 0.41 g. In plants inoculated with *R. intraradices*, *F. mosseae*, and *R. manihotis*, a decrease in the dry biomass of shoot parts was observed with 340 mM NaCl, compared with that seen at 0 mM NaCl. For plants inoculated with *F.*

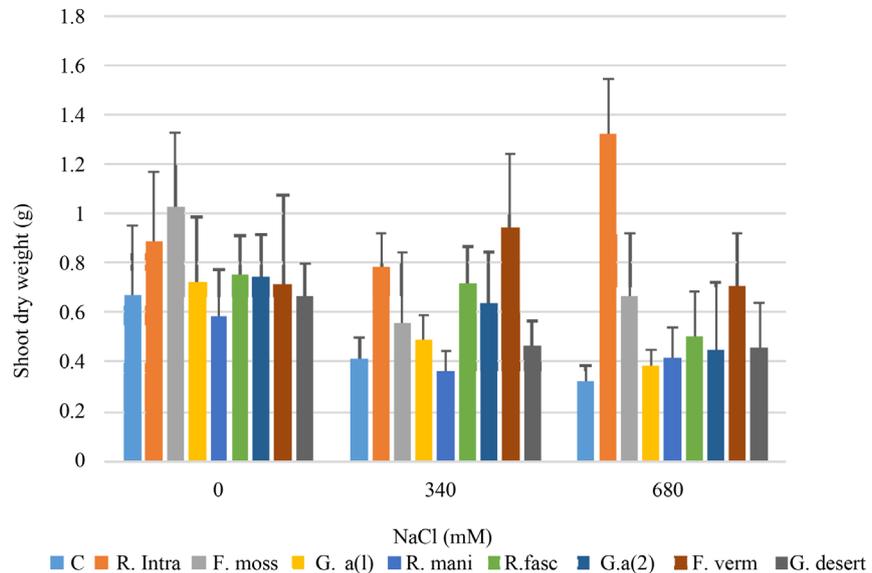


Figure 2. Shoot dry weight biomass of *A. seyal* plants treated with 0, 340 and 680 mM NaCl and inoculated with *R. intraradices* (*R. intra*), *R. manihotis* (*R. mani*), *R. fasciculatus* (*R. fasc*), *F. mosseae* (*F. moss*), *F. verruculosum* (*F. verru*), *G. aggregatum* IR. 27 [*G. a. (1)*], *G. aggregatum* DAOM 227 128 [*G.a (2)*], or *G. deserticola* (*G. desert*), and the non-inoculated control (C) after 18 weeks of culture. Vertical bars represent the standard deviations of six replicates.

verruculosum, greater shoot biomass was observed at 340 mM NaCl (0.94 g), compared with that at 0 mM NaCl (0.71 g), and then a decreased shoot biomass dry weight was noted at 680 mM NaCl (0.70 g). For plants inoculated with *R. intraradices*, shoot biomass was significantly higher (1.50 g) at a NaCl level of 680 mM than at lower NaCl concentrations.

The biomass dry weight of root parts also varied following NaCl treatment (**Figure 3**). The presence of NaCl in the substrate had a depressive effect on the development of non-inoculated roots of *A. seyal*. This was characterized by a decrease in their dry biomass as the level of NaCl increased. In inoculated plants, the effects observed varied according to the AMF species. A steady decline in dry biomass when the level of NaCl increased was noted for plants inoculated with *G. aggregatum* IR. 27, *R. fasciculatus*, and *G. aggregatum* DAOM 227 128. However, a decrease in dry weight was noted when the level of NaCl changed from 0 to 340 mM followed by an increase in the weight of dry root matter at 680 mM NaCl for plants inoculated with *R. intraradices*, *F. mosseae*, *R. manihotis*, *G. deserticola*, and *F. verruculosum*.

3.3. Mortality Rate

Until the application of NaCl (3rd week of culture), the mortality rate of seedlings was zero. At the 6th week of culture (3 weeks after the addition of NaCl), no mortality was observed in plants treated with 0 and 340 mM NaCl; however, at 680 mM NaCl, the mortality rate was 16.7% for plants inoculated with *R. manihotis* and 33.3% for those inoculated with *G. aggregatum* IR. 27, *G. deserticola*,

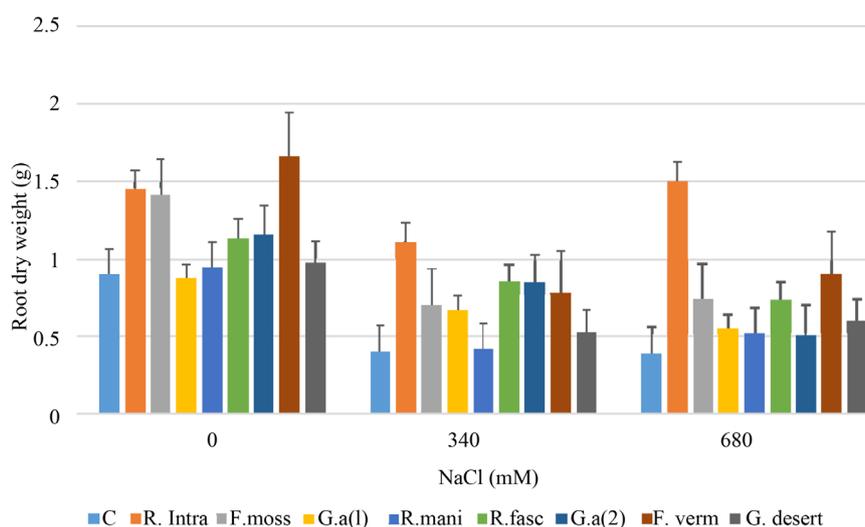


Figure 3. Root dry weight biomass of *A. seyal* plants treated with 0, 340 and 680 mM NaCl and inoculated with *R. intraradices* (*R. intra*), *R. manihotis* (*R. mani*), *R. fasciculatum* (*R. fasc*), *F. mosseae* (*F. moss*), *F. verruculosum* (*F. verru*), *G. aggregatum* IR. 27 [G. a. (1)], *G. aggregatum* DAOM 227 128 [G.a (2)], or *G. deserticola* (*G. desert*), and the non-inoculated control (C) after 18 weeks of culture. Vertical bars represent the standard deviations of six replicates.

and *G. verruculosum*. At the 9th week of culture, mortality rates of 33.3% and 16.70% were observed in control plants treated with 340 mM NaCl and 680 mM NaCl, respectively. At the 18th week of culture, a 16.70% mortality rate was noted in plants inoculated with *G. intraradices* at 680 mM NaCl. At harvest, no mortality was observed among plants inoculated with *F. mosseae*, *R. fasciculatum*, and *G. aggregatum* DAOM 227 128.

3.4. Shoot Mineral Content

The shoot mineral content (N, P, K, and Na) of the plants varied with AMF species inoculated and the NaCl level of the substrate (Table 1). At 0 mM NaCl, the highest contents of N, P, K, and Na were observed in plants inoculated with *F. mosseae*, being, respectively, 21.49, 0.84, 9.11, and 0.92 mg. The lowest levels of N, P, K, and Na were observed in plants inoculated with *R. manihotis*, with respective contents of 12.22, 0.48, 5.17, and 0.52 mg. At 340 mM NaCl, plants inoculated with *F. verruculosum* showed the highest contents of N, P, K, and Na with, respectively, 19.78, 0.77, 8.38, and 0.85 mg. The lowest levels of N, K, and Na were observed in plants inoculated with *R. manihotis*, with 7.56, 3.20, and 0.32 mg. While for P, the lowest levels were noted in plants inoculated with *R. manihotis* (0.29 mg) and *G. deserticola* (0.38 mg), and the controls (0.34 mg). At 680 mM NaCl, plants inoculated with *R. intraradices* and *F. verruculosum* showed the highest levels of P (respectively, 1.08 and 0.58 mg) and K (respectively, 11.77 and 6.30 mg). The highest N contents were observed in plants inoculated with *R. intraradices* (27.76 mg); while for Na, 0.64 mg was noted in plants inoculated with *R. verruculosum*. The lowest levels of N, P, K, and Na

Table 1. Mineral status of shoot parts after 18 weeks of culture.

| NaCl (mM) | Mineral content (mg/plant) | | | | | | | | | | | |
|-----------------|----------------------------|-----------------|-----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| | N | | | P | | | K | | | Na | | |
| 0 | 340 | 680 | 0 | 340 | 680 | 0 | 340 | 680 | 0 | 340 | 680 | |
| Controls | 14.04 ± 5.91ab | 8.61 ± 1.84ab | 6.76 ± 1.27a | 0.55 ± 0.23ab | 0.34 ± 0.07a | 0.26 ± 0.05a | 5.95 ± 2.51ab | 3.65 ± 0.78ab | 2.87 ± 0.54a | 0.60 ± 0.25ab | 0.37 ± 0.08ab | 0.29 ± 0.05a |
| <i>R. intra</i> | 18.62 ± 5.88bc | 16.49 ± 2.84de | 27.76 ± 4.67d | 0.73 ± 0.23bc | 0.64 ± 0.11de | 1.08 ± 0.18d | 7.89 ± 2.49bc | 6.99 ± 1.20de | 11.77 ± 1.98c | 0.80 ± 0.25bc | 0.71 ± 0.12de | 1.19 ± 0.20d |
| <i>F. moss</i> | 21.49 ± 6.34c | 11.73 ± 5.96abc | 13.97 ± 5.29bc | 0.84 ± 0.25c | 0.46 ± 0.23abc | 0.54 ± 0.21bc | 9.11 ± 2.69c | 4.97 ± 2.52abc | 5.92 ± 2.24bc | 0.92 ± 0.27c | 0.50 ± 0.26abc | 0.60 ± 0.23bc |
| <i>G.a.</i> (1) | 15.12 ± 5.29ab | 10.29 ± 2.05ab | 8.03 ± 1.33a | 0.59 ± 0.29ab | 0.40 ± 0.08ab | 0.31 ± 0.05a | 6.41 ± 2.37ab | 4.36 ± 0.87ab | 3.40 ± 0.56a | 0.65 ± 0.24ab | 0.44 ± 0.09ab | 0.34 ± 0.06a |
| <i>R. man</i> | 12.22 ± 4.01a | 7.56 ± 1.74a | 8.74 ± 2.53a | 0.48 ± 0.16a | 0.29 ± 0.07a | 0.34 ± 0.10a | 5.18 ± 1.70a | 3.20 ± 0.74a | 3.70 ± 1.07a | 0.52 ± 0.17a | 0.32 ± 0.07a | 0.37 ± 1.11a |
| <i>R.fasc</i> | 15.79 ± 3.28abc | 14.98 ± 3.20cd | 10.57 ± 3.79abc | 0.62 ± 0.13abc | 0.58 ± 0.12cd | 0.41 ± 0.15abc | 6.69 ± 1.39abc | 6.35 ± 1.36cd | 4.48 ± 1.60abc | 0.68 ± 0.14abc | 0.64 ± 0.14cd | 0.45 ± 0.16abc |
| <i>G.a.</i> (2) | 15.58 ± 3.66abc | 13.27 ± 4.37bcd | 9.38 ± 5.75ab | 0.61 ± 0.14abc | 0.52 ± 0.17bcd | 0.36 ± 0.22ab | 6.60 ± 1.55abc | 5.62 ± 1.85abc | 3.98 ± 2.44ab | 0.67 ± 0.16abc | 0.57 ± 0.19bcd | 0.40 ± 0.25ab |
| <i>F. verru</i> | 14.91 ± 7.60ab | 19.78 ± 6.26e | 14.86 ± 4.45c | 0.58 ± 0.30ab | 0.77 ± 0.24e | 0.58 ± 0.17c | 6.32 ± 3.22ab | 8.38 ± 2.65e | 6.30 ± 1.89b | 0.64 ± 0.33ab | 0.85 ± 0.27e | 0.64 ± 0.19c |
| <i>G.desert</i> | 13.93 ± 2.80ab | 9.66 ± 2.25ab | 9.61 ± 3.78abc | 0.54 ± 0.11ab | 0.38 ± 0.09a | 0.37 ± 0.15abc | 5.90 ± 1.19ab | 4.09 ± 0.95ab | 4.07 ± 1.60abc | 0.60 ± 0.12ab | 0.41 ± 0.10ab | 0.41 ± 0.16abc |

R. intraradices (R. intra); *F. mosseae* (F. moss); *G. aggregatum* IR. 27 [G.a. (1)]; *G. aggregatum* DAOM 227 128 [G.a. (2)]; *R. manihotis* (R. man); *R. fasciculatus* (R. fasc); *G. deserticola* (G. desert); *F. verruculosum* (F. verru); non-inoculated control (C). In the same column, numbers followed by the same letter are not significantly different at $p < 0.05$ (Newman-Keuls test).

were generally observed in control plants and those inoculated with *R. manihotis* and *G. aggregatum* IR. 27.

3.5. Mycorrhization

Mycorrhization of *A. seyal* plants varied according to the AMF isolate inoculated and the NaCl concentration applied to the growth substrate (Table 2). In general, less intense mycorrhization was observed with higher levels of NaCl in the growth substrate. At 0 mM NaCl, the greatest mycorrhization intensities were recorded in plants inoculated with *R. intraradices*, *F. mosseae*, and *G. aggregatum* (IR27), with an average of 32.63%, compared with other treatments, which had an average intensity of 16.55% mycorrhization. At 340 mM NaCl, the analysis of variance revealed no significant differences between the intensities of mycorrhization except for *G. deserticola*, which had the lowest intensity of mycorrhization with 8.13%. At 680 mM NaCl, the highest intensity of mycorrhization was observed in plants inoculated with *R. intraradices* (23.15%) and *R. fasciculatus* (15.28%).

The highest frequency of mycorrhization was observed in plants inoculated with *R. intraradices* at 0 mM NaCl (92.50%). At 340 mM NaCl, the plants inoculated with *R. intraradices*, *F. mosseae*, *G. aggregatum* IR. 27, and *F. verruculosum* showed the highest frequencies of mycorrhization, with an average of 73.75%. At 680 mM NaCl, plants inoculated with *R. intraradices* and *R. fasciculatus* showed the highest rate of mycorrhization, with an average of 82.5%. The lowest rates of mycorrhization were generally noted in plants inoculated with *G. deserticola*.

4. Discussion

The plants of the acacia family appear to have the potential to grow in salty soils [39], with considerable differences in sensitivity to salt stress among species [40] [41]. The tolerance of a plant to salt stress, linked to its genetic potential, could

Table 2. Intensity and frequency of mycorrhization according to the NaCl level in the substrate after 18 weeks of culture.

| NaCl (mM) | Intensity (%) | | | Frequency (%) | | |
|--------------------------|---------------|----------------|-----------------|-----------------|----------------|----------------|
| | 0 mM | 340 mM | 680 mM | 0 mM | 340 mM | 680 mM |
| <i>R. intraradices</i> | 30.88 ± 2.15b | 21.88 ± 1.02b | 23.15 ± 0.96d | 92.50 ± 4.51c | 75.00 ± 2.57c | 82.5 ± 2.88c |
| <i>F. mosseae</i> | 30.00 ± 2.03b | 19.25 ± 1.15b | 12.20 ± 1.02bc | 82.5 ± 2.91abc | 75.00 ± 2.83c | 65.00 ± 2.08bc |
| <i>G. aggregatum</i> (1) | 37.00 ± 2.45b | 18.13 ± 1.11b | 6.5 ± 0.63ab | 85.00 ± 3.02bc | 75.00 ± 2.12c | 55.00 ± 1.16ab |
| <i>R. manihotis</i> | 16.50 ± 1.08a | 13.75 ± 1.06ab | 11.17 ± 1.23abc | 65.00 ± 2.16a | 52.50 ± 1.11ab | 56.67 ± 1.22ab |
| <i>R. fasciculatus</i> | 16.50 ± 1.12a | 19.50 ± 1.15b | 15.28 ± 1.26c | 70.00 ± 1.74ab | 65.00 ± 1.15bc | 82.50 ± 3.02c |
| <i>G. aggregatum</i> (2) | 18.50 ± 1.29a | 18.00 ± 1.14b | 11.5 ± 1.57abc | 80.00 ± 2.91abc | 62.50 ± 1.07bc | 67.50 ± 0.80bc |
| <i>F. verruculosum</i> | 18.75 ± 2.06a | 14.50 ± 0.92ab | 10.25 ± ab | 85.00 ± 3.22bc | 70.00 ± 1.75c | 67.50 ± 0.75bc |
| <i>G. deserticola</i> | 12.50 ± 1.23a | 8.13 ± 0.67a | 5.88 ± 0.58a | 75.00 ± 2.59abc | 42.5 ± 2.52a | 42.5 ± 2.51a |

In the same column, numbers followed by the same letter are not significantly different at $p < 0.05$ (Newman-Keuls test).

be improved by the type of symbiotic microorganisms present in its rhizosphere and by the quality of the interactions that result. In a semi-arid Sahelian environment where numerous biotic and abiotic constraints remain, the contribution of mycorrhizal fungi in an interaction developed with the plant can play a crucial role in helping the plant better cope with abiotic stresses [27] [42] [43]. Many authors have shown the beneficial effect of inoculating plants with AMF, especially when the plants are subjected to salt stress [24] [25] [27] [44] [45] [46]. The results of these studies, in accordance with those of the present study, confirm the hypothesis that mycorrhizal inoculation in general promotes better plant tolerance to salt stress by an improvement in plant survival rate, mineral nutrition, and growth. In the current study, mycorrhiza increased the height, biomass, and viability of *A. seyal* during salt stress, specifically at the seedling stage, which is one of the most sensitive stages for plant growth and development due to growing nutritional needs. According to Giri and Mukerji [42], during salt stress, plants need mycorrhizal fungi not only for adaptation to stress but also for the continuous removal of nutrients during the progressive steps of growth.

Most frequently, AMF observed in saline soils are Glomeraceae species [47] [48] [49] [50] [51]. Interestingly, the fungus *G. deserticola*, isolated from salty soil of *A. seyal*, did not promote better plant development than most of the other species tested. *G. deserticola* did not show an active contribution to mineral nutrition or root colonization in the case of salt stress for this study. The results obtained by Ruiz-Lozano and Azcon [52] on improving mineral nutrition by use of *G. deserticola* in lettuce were not confirmed in this study. Copeman *et al.* [53] noted that the growth of the shoot parts of tomato was increased by inoculation with AMF from non-saline areas and was inhibited by inoculation with AMF from saline areas. The current results confirm those of Cantrell and Linderman [44], who showed that AMF from saline areas were no more able to mitigate the effects of salt stress than those from non-saline areas. They concluded that the ability of different AMF to influence the effects of salinity is not related to a hypothetical adaptation to salinity but rather to a simple reflection of the isolate or to the variation of the ecotype.

The measurement of shoot and root dry biomass showed varied according to the level of NaCl in the substrate and the AMF inoculated. Apart from a few treatments, most of the plants showed decreasing dry weights when the level of NaCl increased. According to Juniper and Abbott [23], the total length of mycorrhizal roots decreases with increasing salinity. Some authors [54] [55] observed that the growth of the hyphae of some AMF is inhibited by increasing concentrations of the NaCl in the substrate, affecting the formation of mycorrhizae and resulting in a change in the frequency and intensity of mycorrhization. The reduction in the dry matter biomass of *A. seyal* seedlings correlated with an increase in NaCl level could be related to a reduction of the mineral element uptake zone of the plant roots and hyphae of the fungi. Jakobsen *et al.* [56] showed

that improved hydromineral nutrition could be linked to the development of the hyphae of mycorrhizal fungi, which would allow plants to better withstand the effects of salt stress by taking essential nutrients to the plant from beyond the root sampling area. The results obtained from seedlings inoculated with *R. intraradices* and *F. verruculosum* indicated that root development of these plants was enhanced by a better stimulation of root system development. This proliferation of the root system was accompanied by a better rate of mycorrhization, which may have led to an improvement in the extraction of mineral elements from the substrate (in particular phosphorus). Poss *et al.* [57] concluded that the tolerance mechanism in onion was mainly attributed to improvements in P nutrition. Similarly, Pfetffer *et al.* [58] noted, “the major effect of mycorrhizae in the removal of sodium is made by means of the phosphorus accumulation”. Nouri *et al.* [59] demonstrated recently that N starvation is partially overruling the suppressive effect of high P nutrition on arbuscule formation. The colonization of seedlings of *A. seyal* by a given AMF could contribute to the improved extraction of mineral elements either by an ability to exclude toxic ions, or by a lowering of the water potential of the plant and/or the AMF in a saline environment, or by a mechanism not yet elucidated.

Many AMF N [60] [61] and Pi specialized transporters [62] involved in the different nutritional pathway has been highlighted. The results obtained in the present study suggest that abiotic changes such as salt stress may modulate different AMF metabolic pathways. The abiotic factor could constitute a stimulus for the plant and/or the fungus, which could induce the activation or the repression of specific genes and lead to variations in transcription factors and physiological responses, helping seedlings to tolerate salt stress. Feddermann *et al.* [30] suggested that differential expression of symbiosis-associated genes among different AMF associations is a phenotypic response to the different fungal and plant genotypes involved and the environment they inhabit; functional diversity is therefore the rule rather than the exception.

This study clearly showed the importance of inoculation for *A. seyal* seedlings in both the presence and absence of NaCl. The effectiveness of symbiotic interactions could be related to the degree of perception of an abiotic factor. For example, a fungus may have no significant effect on the plant for a given level of NaCl but vary the interactive response when the perception of the abiotic factor changes, allowing plants to improve their hydromineral nutrition while lowering the mortality rate. Each species of fungus studied here was characterized by a form of specific interaction with the plant resulting in a diversity of responses associated with the level of stress. This study opens the way for new research perspectives to better understand the regulatory mechanisms of modulating interactions. It would be interesting to study the factors that come into play to allow better tolerance of plants to abiotic stress.

5. Conclusion

AMF can have varying effects in *A. seyal* depending on the level of NaCl in the

substrate. A fungus that is less efficient at promoting growth and survival at low NaCl levels can show very good efficiency when the level of NaCl becomes higher. The variation in the level of NaCl could induce a modification in the activation of the genes regulating the activity of these transporters and consequently the acquisition of nutrients by plants. Future studies on plant-fungus interactions should take into account the variability of responses depending on the type of abiotic stress as well as the genes involved in symbiotic associations in order to achieve the development of more efficient inoculums.

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