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Full Length Article

Arbuscular Mycorrhizal Fungi Enhance Sorghum Plant Growth under Nitrogen-Deficient Conditions through Activation of Nitrogen and Carbon Metabolism Enzymes

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Abstract

Nitrogen (N), one of the most important elements for plant growth, is needed by plants in large quantities. However, this nutrient has limited supply in the soil. Arbuscular mycorrhizal fungi (AMF) are known for their ability to form symbiotic association with plants and transfer the mineral nutrients to the host plants. To validate this hypothesis on sorghum plants, three ecotypes of this cereal (3p4, 3p9 and 4p11) were cultivated with and without AMF under low nitrogen concentration (0.5 m*M* NH_4^+). Growth parameters were determined and key enzymes responsible for nitrogen and carbon metabolisms such as glutamine synthetase (GS), glutamate dehydrogenase (GDH), phosphoenolpyruvate carboxylase (PEPC), isocitrate dehydrogenase (ICDH), malate dehydrogenase (MDH) and asparate aminotransferase (AAT) were measured. For the three sorghum ecotypes, mycorrhizal plants showed a higher plant growth compared to the control plants. The biochemical parameters revealed a significant increase in the nitrogen assimilatory enzymes; GS and GDH in the leaves and roots of mycorrhizal plants. Furthermore, mycorrhizal fungi also appear to have a significant effect on carbon assimilatory enzymes. These enzymes are known to have a cardinal role in the provision of carbon skeletons essential for the assimilation of ammonium and thus, amino acids synthesis. Our study indicates clearly that AMF can be an efficient way to optimize nitrogen uptake and/or assimilation by plants and thus improve the crop yields with lower amount of nitrogen fertilizers. © 2021 Friends Science Publishers

Keywords: Arbuscular mycorrhizal fungi; Carbon metabolism; Enzyme activities; Nitrogen metabolism; *Sorghum bicolor*

Introduction

Appropriate fertilization is a necessity to reach both high yields and quality of crops. Nitrogen (N) is required by plants in large quantities. However, in soil, this element is generally below the levels needed by crops for their optimal growth, the improvement of nitrogen use efficiency, particularly in cereals, is a major goal of crop refinement. Such improved crops would make better use of the nitrogen fertilizer supplied; they would also produce higher yields with better protein content (Yang *et al*. 2012).

Nitrogen is absorbed by plants mainly as nitrate (NO_3^-) or ammonium (NH_4^+). In many plants, most of the nitrate absorbed by the roots is transported to the shoot (Xu *et al.* 2012), where it is reduced first to nitrite (NO_2^-) by the enzyme nitrate reductase (NR) and then to NH_4^+ by nitrite reductase (NiR). As for ammonium, it is directly incorporated into amino acids by the enzymes glutamine synthetase (GS) and glutamate synthase (GOGAT) who plays a central role in nitrogen metabolism (Valadier *et al.* 2008).

During N assimilation, a significant amount of carbon (C) is required to provide C skeleton, including 2-oxoglutarate (2-OG) and oxaloacetate (OAA) used as amino group acceptors (Nunes-Nesi *et al.* 2010). Several enzymes, such PEPC, NADP-ICDH and NAD-MDH could lead to the synthesis of 2-OG (Rademacher *et al.* 2002; Popov *et al.* 2010). Ammonium assimilation in plants occurs principally *via* the GS/GOGAT cycle. Nevertheless, when NH_4^+ is the sole N source, it could be assimilated through another metabolic pathway (Sarasketa *et al.* 2014). Indeed, when NH_4^+ is the unique source of N, GDH were reported to have a primordial role in NH_4^+ assimilation, GDH uses $NH4^+$ and 2-oxoglutarate to produce glutamate (Skopelitis *et al.* 2006; Setién *et al.* 2013).

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Arbuscular mycorrhizal fungi (AMF) are soil borne microorganisms known for their ability to form symbiotic associations with different plant species. Their importance lies in the fact that they increase access to growth-limiting resources by the association of their hyphae to plant roots (Cobb *et al.* 2016). AMF are able to transfer inorganic N to the host plants (Hodge and Fitter 2010). They can also increase the use of different forms of N by plants and have been found to absorb this element directly and to transfer it to the roots of host plant (Govindarajulu *et al.* 2005). Nitrogen assimilated into glutamate and glutamine could be converted to other amino acids, through the enzyme activities of aminotransferase such as AAT, responsible for the formation of aspartate (Torre *et al.* 2014).

Sorghum bicolor (L.) is the 5th most important cereal crop in the world. It is a drought tolerant cereal crop grown in the semiarid tropics of the world. Sorghum could be used as animal feed as it can be used as human food (Prakasham *et al.* 2014). In previous study we have shown that arbuscular mycorrhizal fungi lead to increase in shoots length and biomass in sorghum plants by the enhancement nutrient uptake provided to plants (Kchikich *et al.* 2021). AMF can also increase the use of different forms of N by absorption of this element directly and transfer it to the roots (Govindarajulu *et al.* 2005). Therefore, the aim of this study was to investigate effects of arbuscular mycorrhizal fungi on (i) parameters of growth and chlorophyll content (ii) activities of other enzymes involved in carbon and nitrogen metabolisms such as GS, GDH, ICDH, AAT, PEPC and MDH in roots and shoots of three Moroccan sorghum ecotypes (3p4, 3p9 and 4p11) under nitrogen-limiting conditions. Our overall objective was to determine the possible basis for the response of the mycorrhizal sorghum plants to N deficiency.

Materials and Methods

Plant material and growth

Sorghum seeds were sterilized using 5% of NaOCl for about 15 min and then washed with distilled water. Plants were cultivated in 18 cm plastic pots (3000 cm^3) containing vermiculite as substrate. Twenty seeds per pot of each ecotype were planted. After one week, the plants were thinned to 15 per pot. The plants were grown under controlled conditions at 28°C day/21–22°C night and a photoperiod of 16/8 h (light/dark). Three ecotypes were cultivated in the same conditions. Before sowing, vermiculite was mixed with the AMF (*Glomus intraradices*). Control plants were cultured without AMF. Nitrogen treatment in the form of ammonium sulphate ($(NH_4)_2SO_4$) was provided at 0.5 m*M*. Nitrogen supply was added after one week from the start of the experiment. The shoots and roots were harvested from 5-week-old plants and stored at -80°C until use. The experiment was repeated three times (n = 3) under the same conditions.

Extraction and assay of GS, GDH, NADH-MDH and AAT

Frozen samples were used for extraction by the method described previously (Mrid *et al.* 2018); the leaves and roots were homogenized in 50 m*M* ice-cold phosphate buffer (pH 7.6) containing 14 m*M* β-mercaptoethanol, 1 m*M* Ethylenedia-minetetraacetic acid (EDTA), 1 m*M* phenylmethylsulfonyl fluoride (PMSF), 9.4 μM leupeptin, and 10% (w/v) glycerol. Then the solution was centrifuged at 12,000 g for 20 min and the supernatant was used for determination of enzyme activities. All procedures were performed at 0−4°C.

The GR activity was measured using the transferase assay as described by Shapiro and Stadtman (1970) with some modifications as reported by Mrid *et al.* (2016). The assay mixture consisted of 90 m*M* imidazole-HCl (pH 7.0), 120 m*M* L-glutamine, 3 m*M* $MnCl_2$, 0.4 m*M* ADP, 20 m*M* sodium arsenate, 60 m*M* NH_2OH and the enzyme solution in a final volume of 2.25 mL. The L-glutamine was omitted in the blank test. The reaction was started by adding NH_2OH (prepared freshly, and neutralized to pH 7.0 with NaOH) and incubated at 37°C. The reaction was stopped by adding 0.75 mL of a mixture (1:1:1) of 10% $FeCl_3$•$6H_2O$ (in 0.2 N HCl), 24% TCA and 5% HCl after 15 min. The appearance of γ-glutamyl hydroxamate was measured at 540 nm.

The GDH activity was measured in the aminating direction, as described by Sarasketa *et al.* (2014). The activity was performed in the amination direction at 30°C in reaction buffer containing 100 m*M* Tris-HCl (pH 8), 1 m*M* $CaCl_2$, 13 m*M* α-ketoglutarate, 50 m*M* $(NH_4)_2SO_4$ and 0.25 m*M* NADH. Kinetic activity was determined spectrophotometrically by monitoring NADH at 340 nm. The activity of NAD^+ malate dehydrogenase was assayed by monitoring NADH at 340 nm. The reaction buffer contained 50 m*M* potassium phosphate buffer (pH 7.5), 1 m*M* oxaloacetic acid, 0.25 m*M* NADH and the enzyme solution.

NADH-MDH activity was determined according to the method of Setién *et al.* (2014). The MDH activity was measured by oxidation of NADH and the reduction kinetics of NAD^+ were monitored spectrophotometrically over a period of 3 min at 340 nm. The reaction buffer containing 100 m*M* Hepes-KOH (pH 7.5), 5 m*M* $MgCl_2$, 2 m*M* oxaloacetate and 0.2 m*M* NADH was used. The reactions started by addition of the extracts.

AAT activity was measured following the protocol of Rej (1979) with some modifications (Mrid *et al.* 2018). The activity was measured by coupling oxalacetate production with malate dehydrogenase and NADH and measuring the decrease in absorbance at 340 nm at 30°C in a 1 mL assay mixture containing: Tris-HCl 50 m*M*, pH 7.8, L-aspartate 50 m*M*, 2-oxoglutarate 10 m*M*, NADH 0.1 m*M*, 2U of MDH and 20 μL of roots extract. The reaction was initiated by adding 2-oxoglutarate.

Extraction and assay of PEPC and NADP$^+$-ICDH

Frozen samples were used for extraction by the method described previously (Mrid *et al.* 2018). The supernatant was saturated (60%) with solid ammonium sulphate for 30 min. The saturated supernatant was centrifuged at 12,000 g for 20 min and the resulting pellet was re-suspended in the extraction buffer and used for enzyme assays.

The PEPC activity was carried out following the method of Omari *et al.* (2016). The activity was assayed by coupling to NAD-malic dehydrogenase (MDH) and monitoring NADH oxidation at 340 nm spectrophotometrically in a 1 mL assay mixture containing 100 m*M* Hepes-KOH (pH 7.3), 5 m*M* $MgCl_2$, 0.2 m*M* NADH, 5 U of MDH, 2.5 m*M* PEP (for roots 1 m*M*), 5 m*M* $NaHCO_3$ and leaves or roots extract. One unit of PEPC is the amount of enzyme extract which catalyzes the transformation of 1 μmol substrate per minute at 30°C.

NADP$^+$-ICDH activity monitored as reported by Magalhaes and Huber (1991) with some modifications. The ICDH activity was measured spectrophotometrically by monitoring the oxidation of NADH at 340 nm for 5 min. The assay mixture contains: 50 m*M* potassium phosphate buffer (pH 7.5), 1 m*M* $NADP^+$, 1 m*M* $MnCl_2$ and 4 m*M* isocitrate.

Estimation of protein

Protein content was measured following the method of Bradford (1976). Bovine Serum Albumin (BSA) was used as a protein standard.

Statistical Analysis

Statistical analyses were conducted using the software PASW statistics (v. 18). One-way ANOVA analysis and Tukey's post-hoc tests were conducted to determine significant differences between means ($P < 0.05$).

Results

Effect of AMF on growth parameters

To evaluate the effect of AMF on the three sorghum ecotypes (3p4, 5p3 and 4p11) under nitrogen deficiency, some growth parameters as length and fresh weights were measured. The Table 1 showed that AMF contribute significantly to the growth of the three sorghum ecotypes (5p3, 3p9 and 4p11). In fact, the shoot length of the mycorrhizal plants increased by 36, 45 and 46% for the 3p4, 3p9 and 4p11 ecotypes respectively as compared to control plants. The increase in the fresh weight was about 55, 35 and 44% for these ecotypes compared to the non-mycorrhizal plants.

Table 1: Influence of AM fungal colonization on length and fresh weight (FW) in shoots of three sorghum ecotypes growing at N deficiency condition. (-) Non-inoculated (+) inoculated by *Glomus intraradices*

Ecotype	Shoot length (cm)	Shoot fresh weight (g)
3p4+	28.5 ± 1.29 a	0.28 ± 0.059 a
3p4$^-$	21.00 ± 2.94 bd	0.18 ± 0.031 ab
3p9+	26.88 ± 0.85 c	0.23 ± 0.028 bc
3p9$^-$	18.50 ± 1.29 ce	0.17 ± 0.021 c
4p11+	23.00 ± 1.83 d	0.18 ± 0.025 ac
4p11$^-$	15.75 ± 2.50 c	0.10 ± 0.009 bc

Each value represents the mean of three independent observations with S.D. Different letters indicate significant difference between treatments at 5% level

Effect of AMF on chlorophyll content in leaves of the sorghum plant

Results shown in Fig. 1 indicates that under the N-deficient condition (-N), the total chlorophyll content increased significantly in the mycorrhizal plants. However, the effect of this mycorrhization on the chlorophyll content was not the same. In fact, the 4p11 ecotype was more influenced by the mycorrhization and showed an increase by 58% in the total chlorophyll content, followed by the 3p4 ecotype (25%) and then the 3p9 ecotype (16%).

Effect of AMF on GS and GDH activities in the shoots and roots of sorghum plants

Because the nitrogen source used was ammonium, it was interesting to analyze the enzyme activities of the ammonium assimilatory enzymes; GS and GDH. The Fig. 2 indicates that, regardless of the ecotype, a significant increase in GS activity was noted in both shoots and roots of sorghum in the presence of AMF compared to plants without mycorrhization. In fact, GS activity increased by almost twice for the 3p4 and 3p9 ecotypes and increased by more than twice for the 4p11 ecotype in the shoots of mycorrhizal plants. In roots, GS activity was mainly affected in the 4p11 ecotype followed by the 3p9 ecotype and finally the 3p4 ecotype.

The Fig. 3 indicates that the activity of GDH varied from 0.09 μmol $NADH.min^{-1}.\ g^{-1}$ FW to 0.15 μmol $NADH.min^{-1}.\ g^{-1}$ FW for the shoots of the three ecotypes and from 0.09 μmol $NADH.min^{-1}.\ g^{-1}$ FW at 0.20 m*M* μmol $NADH.min^{-1}.\ g^{-1}$ FW for the roots of the three ecotypes. In the presence of AMF, GDH activity has significantly increased in the roots and shoots of sorghum. Indeed, the activity of GDH increased by 18, 54 and 63% in the shoots, and by 53, 32 and 58 in the roots in the ecotypes 3p4, 3p9 and 4p11, respectively.

Effect of AMF on PEPC and MDH activities in the sorghum shoots and roots

Fig. 4 and 5 showed the activities of PEPC and MDH in shoots and roots of the three sorghum ecotypes grown with or without AMF. Regardless of the ecotype, the increase of

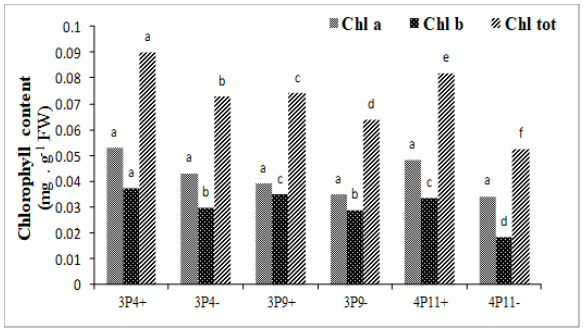

Fig. 1: Influence of AM fungal colonization on chlorophyll content in leaves of three sorghum ecotypes growing at N deficiency condition. (-) Non-inoculated (+) inoculated by *Glomus intraradices*. Each value represents the mean of three independent observations with S.D. Different letters indicate significant difference between treatments at 5% level

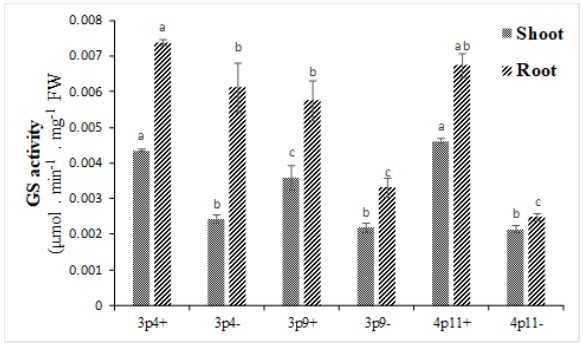

Fig. 2: Influence of AM fungal colonization on glutamine synthetase (GS) activity in shoots and roots of three sorghum ecotypes growing at N deficiency condition. (-) Non-inoculated (+) inoculated by *Glomus intraradices*. Each value represents the mean of three independent observations with S.D. Different letters indicate significant difference between treatments at 5% level

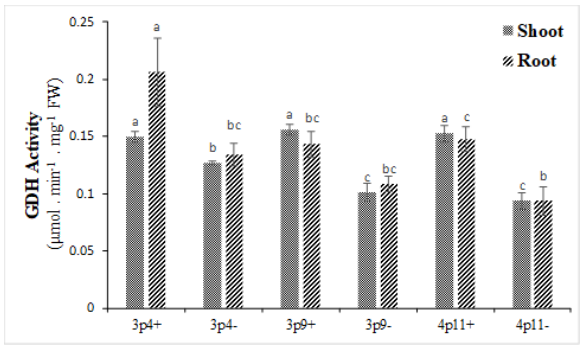

Fig. 3: Influence of AM fungal colonization on glutamate dehydrogenase (GDH) activity in shoots and roots of three sorghum ecotypes growing at N deficiency condition. (-) Non-inoculated (+) inoculated by *Glomus intraradices*. Each value represents the mean of three independent observations with S.D. Different letters indicate significant difference between treatments at 5% level

the activities of these enzymes was significant. For PEPC, the increase of the activity was very remarkable, especially

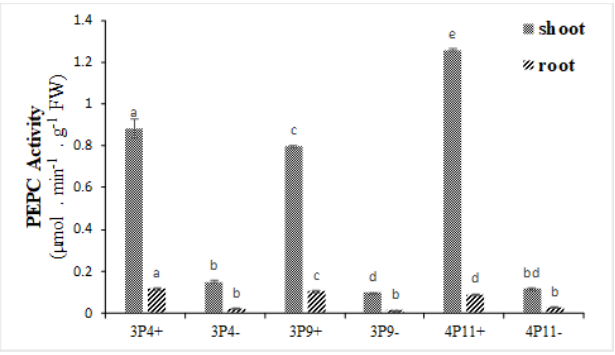

Fig. 4: Influence of AM fungal colonization on phosphoenolpyruvate carboxylase (PEPC) activity in shoots and roots of three sorghum ecotypes growing at N deficiency condition. (-) Non-inoculated (+) inoculated by *Glomus intraradices*. Each value represents the mean of three independent observations with S.D. Different letters indicate significant difference between treatments at 5% level

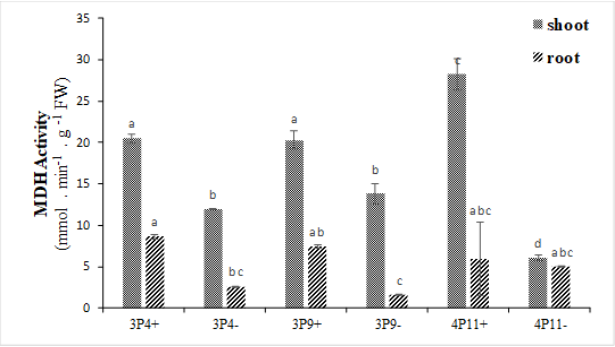

Fig. 5: Influence of AM fungal colonization on malate dehydrogenase (MDH) activity in shoots and roots of three sorghum ecotypes growing at N deficiency condition. (-) Non-inoculated (+) inoculated by *Glomus intraradices*. Each value represents the mean of three independent observations with S.D. Different letters indicate significant difference between treatments at 5% level

in shoots. In fact, the PEPC activity in shoots increased 5-fold for the 3p4 and 3p9 ecotypes, while for the 4p11 ecotype the increase of the activity was 6-fold compared to the control plants. The increase of the MDH activity was also significant. It should be noted here, that for both activities, shoots of the 4p11 ecotype was more influenced by the mycorrhization compared to the two other ecotypes.

Effect of AMF on ICDH and AAT activities in the sorghum shoots and roots

In our study, mycorrhizal ecotypes led to high ICDH activity compared to the control plants. In fact, this activity has almost doubled for the three ecotypes in roots and shoots; however, for the 4p11 ecotype the difference in the enzyme activity between the control plants and the mycorrhizal plants was the much higher (Fig. 6).

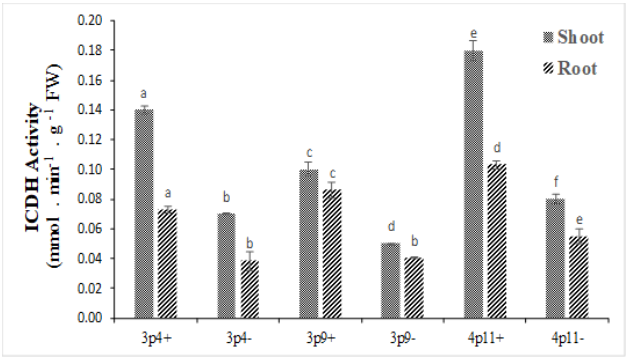

Fig. 6: Influence of AM fungal colonization on isocitrate dehydrogenase (ICDH) activity in shoots and roots of three sorghum ecotypes growing at N deficiency condition. (-) Non-inoculated (+) inoculated by *Glomus intraradices*. Each value represents the mean of three independent observations with S.D. Different letters indicate significant difference between treatments at 5% level

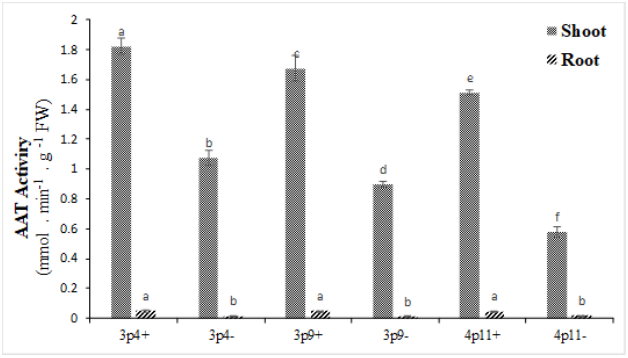

Fig. 7: Influence of AM fungal colonization on aspartate aminotransferase (AAT) activity in shoots and roots of three sorghum ecotypes growing at N deficiency condition. (-) Non-inoculated (+) inoculated by *Glomus intraradices*. Each value represents the mean of three independent observations with S.D. Different letters indicate significant difference between treatments at 5% level

Concerning the AAT activity, Fig. 7 showed the same trend of increase between the mycorrhizal ecotypes and the control plants where a higher effect on the 4p11 ecotype was observed. For this activity, it has been noticed that the activity was more induced in the sorghum shoots compared to the roots (Fig. 7).

Discussion

Sorghum plants inoculated with *Glomus intraradices* showed increased growth, compared to non-mycorrhizal plants. As for sorghum, the effect of AMF on growth enhancement has been described in previous works using different fungal species in combination with strawberry cultivars (Varma and Schuepp 1994). Moreover, Marschner and Dell (1994) reported that the AMF has led in increase of the host plant growth primarily by increasing phosphorus uptake. Other studies have revealed a significant effect of AMF on root development of white clover by endogenous hormone balance. AMF in the soil, which are symbiotic to most terrestrial plants can also enhance plant growth and yield production through increasing the uptake of water and nutrients by the host plant (Wu *et al.* 2011; Shao *et al.* 2018).

Nitrogen is an essential constituent of chlorophyll. An adequate supply of N might result in high photosynthetic activity and vigorous vegetative growth (Kafle and Sharma 2015). Mitova *et al.* (2017) have found that the chlorophyll content in two varieties of lettuce was affected significantly by the mycorrhizal fungi inoculation, but much higher values were observed in one of these varieties compared to the other. The increase of the chlorophyll content by the mycorrhizal fungi inoculation might be due to the activated synthesis of free amino acids triggering the chain of chlorophyll biosynthesis (Smolov and Semenova 2008). Qin *et al.* (2017) reported that high chlorophyll content in mycorrhizal plants may be responsible for the increment in nutrients uptake such us N, P and Mg.

Nitrogen (N) is among the most important macro-nutrients significantly affecting plant growth and yield production. Glutamine synthetase is one of the key enzymes responsible for the assimilation of inorganic N. It catalyzes the formation of glutamine. This metabolite will provide N groups for the biosynthesis of all nitrogenous compounds of the plant. In the present study we observed increased activity of GS in roots and shoots of the sorghum plants under Arbuscular mycorrhizal (AM) fungus. Mitova *et al.* (2017) have obtained the same trend of changes regarding GS activity with a differential response between the different tested varieties of lettuce. In another study, three AMF (*Glomus intraradices*, *G. etunicatum* and *G. mosseae*) enhanced the GS activity in the roots of maize (Deng *et al.* 2009). According to these authors it has been indicated that AMF plays a significant role in NH_4^+ utilization of the host plants.

Among the enzymes having the capacity to catalyse the assimilation of NH_4^+ into organic molecules, we can cite the glutamate dehydrogenase (GDH). In fact, it was reported that NADH-GDH may incorporate ammonium in glutamate under stress conditions (Skopelitis *et al.* 2006; Masclaux-Daubresse *et al.* 2010; Setién *et al.* 2013; Omari and Nhiri 2015). Saito (1994) reported that increased GDH activity is associated with plant mycorrhization.

The enhanced activities of glutamine synthetase, and glutamine synthase in the roots and shoots of mycorrhizal corn indicate that the absorbed NO_3 by AM hypha can be directly transferred to the root cells for further utilization and incorporation into the organic structures. Such enzymatic alterations can also enhance plant resistance to drought stress. This indicates that in addition to the direct effects of AM fungi on the alleviation of stresses such as drought, their indirect effect such as absorbing inorganic N

can also contribute to the alleviation of stress. AM fungi are able to alter plant physiological and morphological properties in a way by which plant can handle the stress (Miransari 2011).

The increase of the enzymatic activities of GS and GDH in the roots and shoots of the three sorghum ecotypes indicates that ammonium might first be concentrated by AMF and then translocated to the root cells for use and incorporation into organic structures. The results found by Nakmee *et al.* (2016) confirm the results that we obtained. In fact, these authors revealed that the AMF significantly increased the percentage of N in shoots and the total N uptake in shoots and roots of sorghum. In another study conducted by Govindarajulu *et al.* (2005) using stable isotope labelling experiments reported that the inorganic N absorbed by the AMF outside the roots is metabolized to form amino acids. Amino acids are then translocated to the intraradical mycelium as arginine. Arginine is then transferred to the plant without carbon. Ammonia generated from arginine catabolism is translocated to the host *via* ammonia channels. These results could explain the increase of the enzymes GS/GDH activities in the plants.

To our knowledge, this is the first study aiming to determine the effect of plant mycorrhization on the carbon metabolism enzymes under N deficiency conditions in sorghum plant. In a study conducted by Hashem *et al.* (2015), the authors showed that the AMF can alleviate the decease of carbon assimilation-related enzyme activities, such as PEPC, induced by salt stress. In our study, the increase in the PEPC and MDH activities may be related to the increase in the N assimilation-related enzyme activities (GS and GDH). There are many reports of increased PEPC activity with NH^{4+} *versus* nitrogen nutrition although other works show that NO_3^- supply can stimulate PEPC activity (Champigny 1995). In fact, the accumulation of amino acids in the roots and leaves requires an adequate amount of keto acids, particularly the 2-oxoglutarate and oxaloacetate. These carbon skeletons originate from the tricarboxylic acid cycle (TCA). Both PEPC and MDH have been shown to fulfil a central role in the replenishment of the TCA. Thus, the increase in PEPC and MDH activities could be essential for the supply of carbon compounds required for the synthesis of amino acids and thus, proteins (Mrid *et al.* 2018). In the study conducted by Chen *et al.* (2017), the authors showed that mycorrhizal fungi of the genera *Funneliformis*, *Claroideoglomus*, *Rhizophagus* and *Diversispora* were responsible for an increase in the stomatal conductance and the intensity of CO_2 assimilation in cucumber plants.

In the literature, isocitrate dehydrogenases and aspartate aminotransferases were reported to have a direct role in the furniture of key organic acids for the assimilation of ammonium (Hodges *et al.* 2003). It was stated that 95% of the total ICDH activity in green tobacco leaves was attributed to the cytosolic form of the ICDH (Gálvez *et al.* 1994) and that this enzyme is the predominant isoform in several plants (Fieuw *et al.* 1995; Gallardo *et al.* 1995; Palomo *et al.* 1998). It has been proposed that this cytosolic ICDH may play a major role in the production of 2-oxoglutarate for amino acid synthesis (Mrid *et al.* 2017). Boiffin *et al.* (1998) showed that the activity of $NADP^+$-ICDH increased in the roots of *Eucaluptus globulus* subsp. *Bicostata* during colonization by an ectomycorrhizal fungus.

Mycorrhizal fungi are able to enhance the uptake of N from NH_4^+ fertilizers and carrying it to their host plants (Ames *et al.* 1983; Johansen *et al.* 1993). Chambers *et al.* (1980) have shown that amides and amino acids, particularly; asparagine and aspartate could be higher in exudates from ammonium fed plants. We can suggest that the higher AAT activity in mycorrhizal plants can be correlated with its role in metabolizing glutamate, resulting from NH_4^+ assimilation to aspartate that may be used for the biosynthesis of other amino acids (Forde and Lea 2007).

Conclusion

In present work, we showed also that AMF had a positive effect on plant growth. We showed also that the results of the growth parameters are in line with the results of the carbon and nitrogen metabolism enzyme activities. In fact, the role of these enzymes is strongly related to the synthesis of organic compounds required for nitrogen assimilation and thus amino acids and protein synthesis a process required for plant growth and development. These results could have an important socio-economic and ecological impact because they show a clear increase in the efficiency of nitrogen utilization for the mycorrhizal plants under low nitrogen inputs. This would save the costs associated with the input of nitrogen fertilizer and will also reduce any pollution related to its use.

Author Contributions

Anass Kchikich and Reda Ben Mrid conceived and designed the experiments; Anass Kchikich, Imad Kabach, and Reda Ben Mrid performed the experiments; Anass Kchikich, Imad Kabach, Reda Ben Mrid, Mohamed Nhiri, and Redouane El Omari analyzed the data and prepared the document; Mohamed Nhiri and Redouane El Omari, supervised the work.

Conflict of Interest

There are no conflicts to declare.

Data Availability

The data presented in this study are available on request from the corresponding author.

Ethics Approval

There are no researches conducted on animals or humans.

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