Effect of Mycorrhization on Growth and Enzymes involved in Carbon/Nitrogen interaction in Sorghum Plants

Kchikich Anass[1], Ben Mrid Reda[1]*, Kabach Imad[1], El Omari Redouane[1,2], Bouargalne Youssef[1], Amakran Amina[1] and Nhiri Mohamed[1]
1. Laboratory of Biochemistry and Molecular Genetics, Faculty of Science and Technology of Tangier, Abdelmalek Essaâdi University, BP 416, 90000 Tangier, MOROCCO
2. Laboratory of Sustainable Agriculture Management, Higher School of Technology (EST) Sidi Bennour, Chouaib Doukkali University, El Jadida, MOROCCO
*rbenmrid@gmail.com

Abstract
Improved crop yield is proportional to nitrogen (N) fertilizer applied to agricultural land. However, N fertilizers are also major source of environmental pollution mainly in soils, water and the atmosphere. Determining the optimal amount of N fertilizer needed for crop growth is therefore essential. Arbuscular mycorrhizal fungi (AMF) characterized by their capacity to provide mineral nutrients are also exploited by the soil beyond the zone of influence of the plant root and by an important role in reducing the amount of N fertilizer. Thus, three Moroccan sorghum ecotypes (5p3, 3p9 and 4p11), cereal with agronomic and economic interest, were cultivated with or without AMF. Growth parameters were measured and key enzymes responsible for carbon/nitrogen interaction such as glutamine synthetase (GS), glutamate dehydrogenase (GDH), $NADP^+$-Isocitrate dehydrogenase (ICDH), and NAD^+-malate dehydrogenase (MDH) have been determined.

In the three ecotypes, mycorrhizal plants showed a longer plant length compared to control plants. The biochemical parameters showed a significant increase in GS and ICDH activity in the leaves and roots of mycorrhizal plants. However, mycorrhizal fungi appear to affect the activity of GDH and MDH only in the root of sorghum plants. AMF can be an effective way to optimize nitrogen uptake by the plant and thus improve crop yields with lower amounts of nitrogen fertilizers.

Keywords: *Sorghum bicolor*, Arbuscular mycorrhizal fungi, Carbon/nitrogen interaction, Crop, Yields.

Introduction
Sorghum bicolor (L.) is a cereal belonging to the family of *Poaceae* which is commonly known as sorghum. This cereal is one of the most important crops in Africa, Asia and Latin America. It is cultivated in semi-arid and tropical areas because of its adaptation to different environmental stresses including drought stress[1]. In developing countries and particularly in West Africa, the demand for sorghum increases every year. This is not only due to population growth, but also due to the policies of these countries to improve their treatment and industrial use[7].

One of the keys to enhance sorghum production could be to improve mutualistic partnerships with soil beneficial microbes such as arbuscular mycorrhizal fungi (AMF), a source of biological fertilization. Structural relationships include both the fungus and its host root system; they are considered as nutritional symbiosis because they are the main suppliers of different nutrients for the majority of terrestrial plants.[16]

AMF can increase access to growth-limiting resources by their hyphae associated with plant roots that can extend the reach of root systems[6]. In this symbiosis, fungi provide the host root with nutrients and water in exchange for carbon. In addition to plant mineralization, they can also improve disease resistance, water use efficiency, soil structure, and beneficial microbial activity in natural ecosystems[17].

Nitrogen is frequently a limiting factor for plant growth and development. The uptake of N by mycorrhizal fungi may, therefore, be an important route of N absorption by the plant. AMF can also increase the use of different forms of N by plants and have been found to absorb this element directly and transfer it to the roots[12].

Therefore, the aim of this study was to investigate effects of arbuscular mycorrhizal fungi on (i) parameters of growth (ii) activities of other enzymes involved in carbon and nitrogen metabolisms such as Glutamine synthetase (GS), Glutamate dehydrogenase (GDH), $NADP^+$-Isocitrate dehydrogenase (ICDH) and NAD^+-malate dehydrogenase (MDH) in roots and leaves of three Moroccan sorghum ecotypes (5p3, 3p9 and 4p11).

Material and Methods
Sorghum seeds (*Sorghum bicolor*) were sterilized with 5% of NaOCl for 15 minutes and washed thoroughly with sterile water. Plants were then cultivated in 18-cm plastic pots (3000 cm^3) containing vermiculite. Twenty seeds per pot of each ecotype were planted. After one week, the plants were thinned to 15 per pot. The plants were grown in a controlled environment chamber at 28°C day/21-22°C night with a photoperiod 16/8 h (light/dark). The ecotypes were

cultivated in the same conditions and received the same treatments. Before sowing, vermiculite was mixed with the AMF, *Glomus intraradices*. Control plants are cultured in the absence of AMF. Nitrogen treatment in the form of nitrate (NO_3^-) is provided at 5 mM. Nitrogen supply was added after one week from the start of the experiment.

After 5-week old, plants from each ecotype were harvested and divided into separate leaves and roots fractions. Fresh weights of leaves and roots were weighed and lengths were measured. The samples were then stored at -80°C until analysis (determination of enzymatic activities). The experiment was repeated three times (n = 3) under the same conditions.

Frozen samples were extracted into 4 volumes of a 50 mM Tris-HCl buffer (pH 8) containing 14 mM β-mercaptoethanol, 10% glycerol (v/v), 4 µg/ml leupeptin, 10 mM glutamate, 1 mM EDTA and 0.5 mM $MgSO_4$. The homogenate was centrifuged at 20,000xg for 30 minutes at 4°C to obtain a clarified supernatant.

Glutamine synthetase activity was measured using the transferase assay as described by Shapiro and Stadtman[23]. The test mixture consisted of 90 mM imidazole-HCl (pH 7.0), 120 mM L-glutamine, 3 mM $MnCl_2$, 0.4 mM ADP, 20 mM potassium arsenate, 60 mM NH_2OH and the enzyme solution. The reaction was started by adding NH_2OH (freshly prepared and neutralized with NaOH) and incubated at 37°C for 20 minutes. At the end of the incubation period, 0.75 ml of a mixture (1:1:1) of 10% $FeCl_3 \cdot 6H_2O$ (in 0.2 N HCl), 24% TCA and HCl at 5% were added to stop the reaction. The samples were centrifuged at 13200 g for 5 minutes and the absorbance of γ-glutamyl-hydroxamate was measured at 540 nm.

Glutamate dehydrogenase activity was performed in the amination direction at 30°C in reaction buffer containing 100 mM Tris-HCl (pH 8), 1 mM $CaCl_2$, 13 mM α-ketoglutarate, 50 mM $(NH_4)_2SO_4$ and 0.25 mM 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 mM potassium phosphate buffer (pH 7.5), 1 mM oxaloacetic acid, 0.25 mM NADH and the enzyme solution.

For the $NADP^+$- Isocitrate Dehydrogenase**,** the leaves were extracted in 4 volumes of 100 mM Tris-HCl buffer, pH 8 containing 10 mM $MgCl_2$, 10% (v/v) glycerol, 14 mM ß-mercaptoethanol, 1 mM PMSF, 1 mM EDTA, 1 mM EGTA and 4µg ml^{-1} leupeptin. The homogenates were centrifuged at 12000g for 15 minutes at 4°C. The supernatant was then saturated (60%) with solid ammonium sulfate for 30 minutes; the saturated supernatant was centrifuged again under the same conditions and the resulting pellet was resuspended in the extraction buffer and used for enzymatic assays.

The activity of ICDH was measured spectrophotometrically by monitoring the oxidation of NADH at 340 nm for 5 minutes at 30°C. The NADP-ICDH reaction was measured at 30°C in a reaction buffer containing 50mM potassium phosphate buffer (pH 7.5), 1mM $MnCl_2$, 1mM $NADP^+$ and 4mM isocitrate.

The data are mean values ± SD (standard deviation). Results were subjected to one-way Analysis of Variance (ANOVA) followed by the Tukey test using SPSS statistics (version 18). The differences were considered to be significant when $P<0.05$.

Results and Discussion

To evaluate how AMF affects three sorghum ecotypes (5P3, 3P9 and 4P11), some growth parameters as length and fresh weights were measured. Table 1 shows the effect of AMF on the growth parameters of sorghum shoots. From these results, we can observe that AMF contributed in a beneficial way to the growth of the three sorghum ecotypes (5p3, 3p9 and 4p11). In fact, the length and the fresh weight of the aerial part of the mycorrhizal plants were significantly greater compared to the control plants.

On other hand, AMF showed also a significant increase in fresh weight which was higher in mycorrhizal plants by 68% for the 4P11 ecotype, 47% for the 5P3 ecotype and only 43% for the 3P9 ecotype compared to the control plants. AMF were considered as the most ubiquitous soil microorganisms, forming mutualistic associations with 80-90% of vascular plant species in the world's ecosystems.

Table 1

Effect of Arbuscular mycorrhizal fungi (AMF) supply (+) on length and fresh weight in shoots of three Moroccan *Sorghum bicolor* ecotypes (5p3, 3p9 and 4p11). Each value represents the mean of three independent observations with S.D. Different letters indicate significant differences at p<0.05 when compared to control.

Ecotype	Length (cm)	Fresh Weight (g)
5p3+	27.75 ± 2.50^a	0.270 ± 0.033^a
5p3-	22.75 ± 1.70^b	0.184 ± 0.030^b
3p9+	29.37 ± 1.10^a	0.282 ± 0.016^a
3p9-	24.75 ± 1.25^{ab}	0.198 ± 0.011^b
4p11+	26.50 ± 2.08^{ab}	0.284 ± 0.026^a
4p11-	16.62 ± 1.10^c	0.169 ± 0.025^b

Previous research has indicated that inoculation with AMF enhances nutrient uptake of phosphorus and nitrogen and promotes sorghum growth[18].

Ammonium generated from nitrate reduction by the successive action of nitrate reductase and nitrite reductase absorbed directly from the medium or generated by catabolism of amino acids is mainly assimilated by the Glutamine synthetase/Glutamate synthase (GS/GOGAT) pathway[3]. Previous works on transgenic plants suggested that alteration or overexpression of GS could accelerate plant development[15]. In the present study, figure 1 indicates that regardless of the ecotype, there was a significant increase in GS activity in the leaves and roots of mycorrhizal sorghum compared to the control plants.

For the plants cultivated with AMF, the GS activity increased by 67, 181 and 110% in leaves, and 247%, 8% and 153% in roots for 5P3, 3P9 and 4P11 respectively. The effect of the mycorrhization was not the same for the three ecotypes. In fact, for the 3p9 and 4p11 ecotypes, the effect was more pronounced in leaves, however, for the 5p3 ecotype, the mycorrhization affected mainly the GS activity of the root plants. Our GS activity results are in line with the work conducted by Govindarajulu et al[12] and Bücking et al[4].

Govindarajulu et al[12] showed that AMF can absorb and transfer significant amounts of nitrogen to their host plants. The mycorrhizal association can also increase the use of different forms of nitrogen by plants and can allow direct absorption of nitrogen to the host roots which may explain the increase of the GS activity in the mycorrhizal plants.

Among the enzymes having the capacity to catalyse the reaction of incorporation of ammonium into organic molecules, we can mention the Glutamate dehydrogenase (GDH). It has been reported that NADH-glutamate dehydrogenase may incorporate ammonium in glutamate in response to high levels of ammonium under stress[14,22,24]. Smith et al[25] found no consistent effect of mycorrhizal infection on GDH activity in mycorrhizal *Trifolium subterraneum* roots. However, Saito[20] reported that increased GDH activity is associated with plant mycorrhization. According to Bücking[4], the reduction of NO_3^- in non-mycorrhizal plants, occurs mainly in the leaves whereas in mycorrhizal plants, NO_3^- is mainly reduced in the roots. These results are consistent with our results on GDH activity.

In fact, results of figure 2 indicate that GDH activity ranged from 0.054 µmol NADH.min-^{1}.g-^{1} FW to 0.19 µmol NADH.min-^{1}.g-^{1} FW for the three ecotype shoots and from 0.08µmol NADH.min^{-1}.g^{-1} FW to 0.19µM mmol NADH.min^{-1}.g^{-1} FW for the three ecotype roots. In presence of AMF, there was a significant increase in GDH activity in sorghum roots. Indeed, the GDH activity increased by 55, 138 and 111% for 5P3, 3P9, and 4P11 respectively. However, a remarkable decrease in shoots GDH activity was observed for the three ecotypes. In roots, maximal activities were found in the 4P11 and 3P9 ecotypes treated by AMF (0.19 µmol NADH.min^{-1}.g^{-1} FW).

It can be concluded from these results that the high activity of GDH in the roots of mycorrhizal plants indicates that glutamate is mainly produced in the roots of mycorrhizal plants, however, in control plants, the main production of glutamate occurs in the leaves. This can be explained by the fact that the expression of the fungal GDH gene controls the expression of the GDH gene in the leaves. When the plant is colonized by a fungal strain, the expression of leaves GDH is repressed, but the expression increases in the colonized roots.

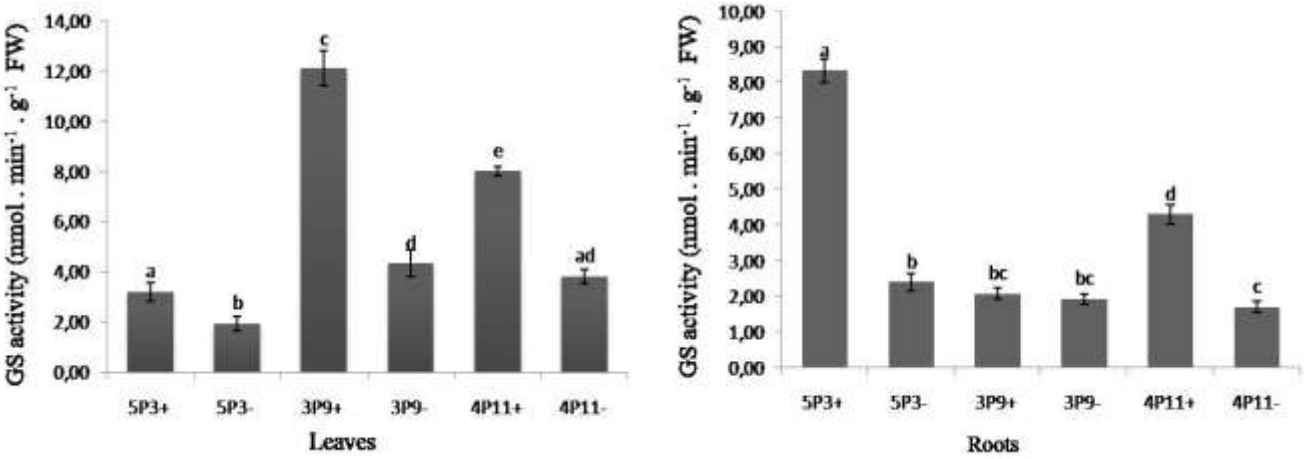

Figure 1: Glutamine Synthtase activity (GS) in the leaves and the roots of three Moroccan *Sorghum bicolor* ecotypes (5p3, 3p9 and 4p11) grown with (+) Arbuscular mycorrhizal fungi (AMF) supply. Each value represents the mean of three independent observations with S.D. Different letters indicate significant differences at p<0.05 when compared to control (-).

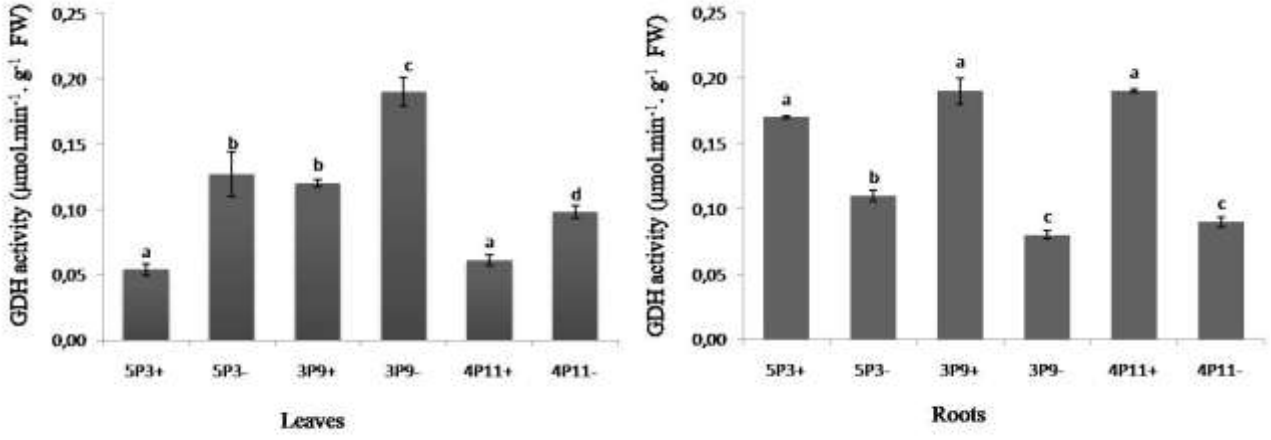

Figure 2: Glutamate dehydrogenase activity (GDH) in the leaves and the roots of three Moroccan *Sorghum bicolor* ecotypes (5p3, 3p9 and 4p11) grown with (+) Arbuscular mycorrhizal fungi (AMF) supply. Each value represents the mean of three independent observations with S.D. Different letters indicate significant differences at $p<0.05$ when compared to control (-).

The increase of GS activity in the roots and leaves and GDH activity in the roots of the three sorghum ecotypes may indicate that the NO_3^- absorbed by AMF can be directly transferred to root cells for use and incorporation into organic structures. It was reported that such enzymatic alterations can also enhance plant growth[17].

The results found by Nakmee et al[18] confirm the results of this study. Indeed, these authors found that fungal inoculation of AMF significantly increased the percentage of nitrogen in shoots and the total nitrogen uptake in shoots and roots of sorghum. This characteristic could have an important socio-economic and ecological impact because mycorrhization could increase the efficiency of nitrogen utilization and thus significantly reduce nitrogen inputs to the fields. This would save the costs associated with the input of this fertilizer and will also reduce any pollution related to its use.

In the literature, isocitrate dehydrogenases and aspartate aminotransferases are the two main enzymes implicated in synthesis of key organic acid for the uptake of plant ammonium[13]. It was reported that the cytosolic form of the ICDH is responsible for 95% of the total activity of ICDH in green tobacco leaves[10], and is the predominant isoform in tomato, potato and the only detectable form in pine cotyledons[8,9,19]. It has been proposed by Chen and Gadal[5] that cytosolic ICDH may play a major role in the production of 2-oxoglutarate for amino acid synthesis. In our study, the $NADP^+$-ICDH activity was positively affected by AMF in both leaves and roots as shown in figure 3.

The activity was enhanced by 33, 67, and 29% in leaves and to 60%, 75%, and 160% in roots for the 5P3, 3P9, and 4P11 ecotypes respectively. The AMF seemed to affect effectively the $NADP^+$-ICDH activity in the leaves and the roots of 3P9 ecotype. For the 4P11 ecotype, the mycorrhization affected mainly the $NADP^+$-ICDH activity in roots, however, the variation of the activity in 5P3 was the lowest compared to the other ecotypes.

These results are in accordance with the results obtained by Boiffin et al[2], who worked on *Eucaluptus globulus* subsp. *Bicostata* whose activity of NADP-ICDH increased in the roots during colonization by the ectomycorrhizal fungus. These results are in line with our previous conclusion that AMF improved the growth and nutrient uptake of nitrogen[18].

Figure 4 shows the NADH-MDH activity in plants grown with and without AMF. Regardless of the ecotype, there was a significant increase in this activity in sorghum roots. In fact, the activity in roots increased by 63, 224 and 133% for 5P3, 3P9 and 4P11 respectively. The increase was more pronounced in the 3P9 and 4P11 ecotypes compared to the 5P3.

Moreover, the NADH-MDH in shoots did not show any significant differences between the mycorrhizal plants and the control plants. The maximum activity in the mycorrhizal plants was obtained in the roots of the 4p11 ecotype.

MDH catalyzes the interconversion of malate and oxaloacetate (OAA) coupled with reduction/oxidation of NAD pool organelles[27]. Cytosolic NAD^+ -MDH has been reported to catalyze malate formation from OAA. This malate enters the mitochondria where mitochondrial MDH catalyzes the conversion of malate to OAA[11,26]. The formed OAA is then used in the TCA cycle. In this way, increasing NAD^+-MDH under nitrogen fertilizer seems to be essential for growth processes as they allow the TCA cycle to continue[21]. Saito[20] obtained similar results; indeed, these authors reported that the increase in MDH activity is associated with mycorrhizal onion.

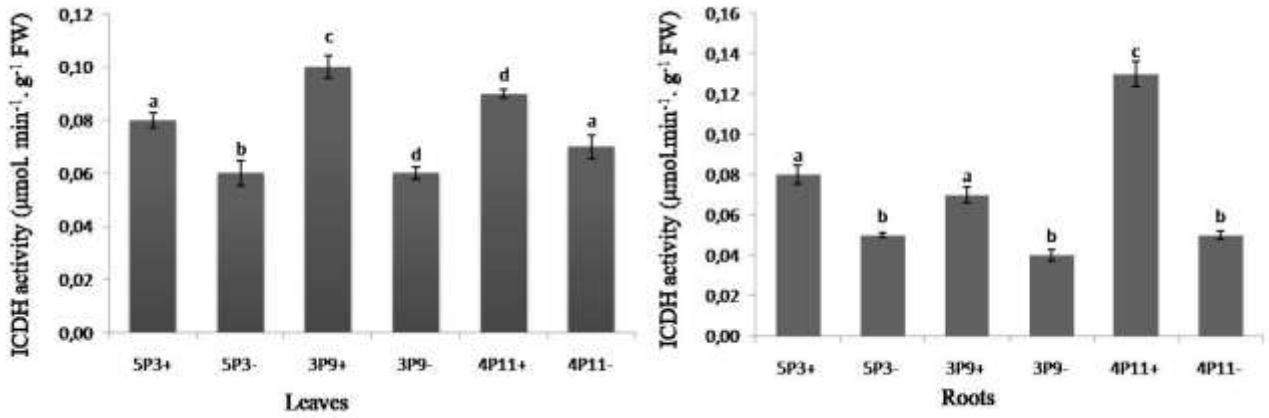

Figure 3: NADP$^+$-Isocitrate dehydrogenase (ICDH) in the leaves and the roots of three Moroccan *Sorghum bicolour* ecotypes (5p3, 3p9 and 4p11) grown with (+) Arbuscular mycorrhizal fungi (AMF) supply. Each value represents the mean of three independent observations with S.D. Different letters indicate significant differences at $p<0.05$ when compared to control (-).

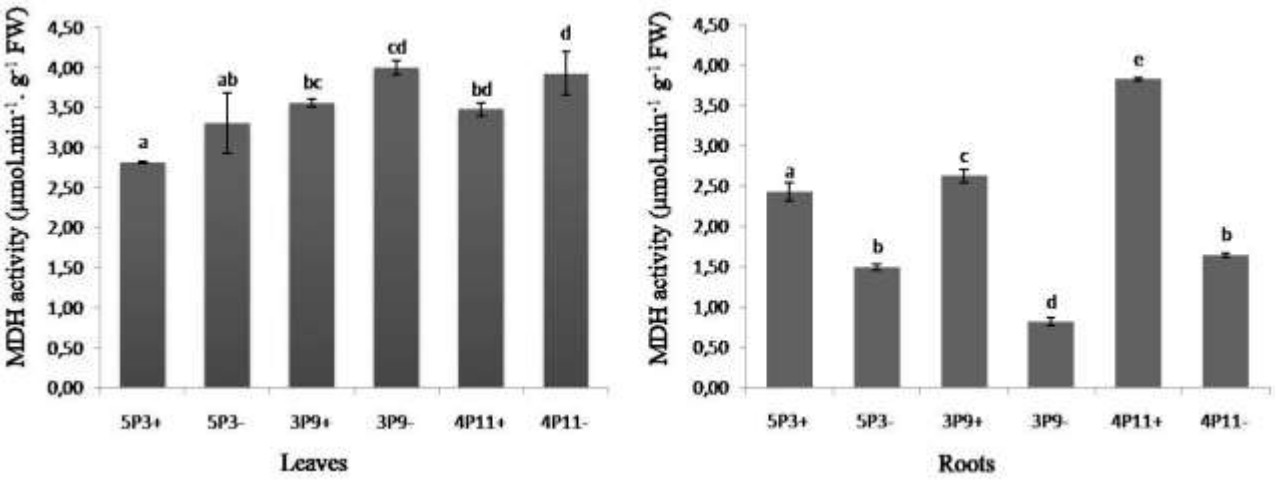

Figure 4: NAD$^+$-malate dehydrogenase (MDH) in the leaves and the roots of three Moroccan *Sorghum bicolour* ecotypes (5p3, 3p9 and 4p11) grown with (+) Arbuscular mycorrhizal fungi (AMF) supply. Each value represents the mean of three independent observations with S.D. Different letters indicate significant differences at $p<0.05$ when compared to control (-).

These results can be explained by the fact that the roots are the major organs of synthesis of the carbon skeletons and it is possible that the AMF induced the strong MDH activity in the roots to synthesize the carbon skeletons which will be transferred thereafter to the leaves. On the other hand, the large availability of carbon skeletons, originating in the roots and transported to the leaves probably induced the decrease of the MDH activity in the leaves.

Conclusion

In this study, we showed that arbuscular mycorrhizal fungi had a significant effect on plant growth and led to an increase in shoots length and biomass. The results of the morphological parameters are correlated with the results of the enzymatic activities studied, since the role of the enzymes studied is strongly related to the synthesis of various organic compounds which leads to the accumulation of biomass, then shoot growth.

Inoculation of the studied ecotypes by Arbuscular mycorrhizal fungi significantly improved the growth of these ecotypes. This result is due to the enhanced nutrient uptake provided to plants and therefore arbuscular mycorrhizal fungi can play an important role in improving organic agriculture and can present environmentally friendly biofertilizers.

References

1. Ben Slima S., Ktari N., Trabelsi I., Moussa H., Makni I. and Salah R.B., Purification, characterization and antioxidant properties of a novel polysaccharide extracted from Sorghum bicolor (L.) seeds in sausage, *Int. J. Bio. Macrom*., **106**, 168-178 **(2018)**

2. Boiffin V., Hodges M., Galvez S., Balestrini R., Bonfante P., Gadal P. and Martin F., Eucalypt NADP-Dependent Isocitrate Dehydrogenase cDNA Cloning and Expression in Ectomycorrhizae, *Plant Physiol*ogy, **117**, 939-948 **(1998)**

3. Brugiere N., Suzuki A. and Hirel B., Ammonium assimilation, In Morot-Gaudry J.F., ed., Nitrogen Assimilation by Plants, Physiological, Biochemical and Molecular Aspects, Science Publishers, Inc, 71-94 **(2001)**

4. Bücking H. and Kafle A., Role of Arbuscular Mycorrhizal Fungi in the Nitrogen Uptake of Plants: Current Knowledge and Research Gaps, *Agronomy*, **5**, 587-612 **(2015)**

5. Chen R.D. and Gadal P., Do mitochondria provide the 2-oxoglutarate needed for glutamate synthesis in higher plant chloroplasts?, *Plant Physiol. Bioch.,* **28**, 141-145 **(1990)**

6. Cobb A.B., Wilson G.W., Goad C.L., Bean S.R., Kaufman R.C., Herald T.J. and Wilson J.D., The role of arbuscular mycorrhizal fungi in grain production and nutrition of sorghum genotypes: Enhancing sustainability through plant-microbial partnership, *Agr. Eco. Envi.*, **233**, 432-440 **(2016)**

7. Dicko M.H., Gruppen H., Traoré A.S., Voragen A.G. and Van Brekel W.J., Sorghum grain as human food in Africa: relevance of content of starch and amylase activities, *Afr. J. Biot*., **5**, 384-395 **(2006)**

8. Fieuw S., Müller-Rober B., Cálvez S. and Willmitzer L., Cloning and Expression Analysis of the Cytosolic $NADP^+$-Dependent lsocitrate Dehydrogenase from Potato, *Plant Physiol*., **107**, 905-913 **(1995)**

9. Gallardo F., Miginiac-Maslow M., Sangwan R.S., Decottignies P., Keryer E., Dubois F., Bismuth E., Galvez S., Sangwan-Norreel B., Gadal P. and Crétin C., Monocotyledonous C_4 $NADP^+$-malate dehydrogenase is efficiently synthesized, targeted to chloroplasts and processed to an active form in transgenic plants of the C3 dicotyledon tobacco, *Planta*, **197**, 324-332 **(1995)**

10. Gálvez S., Bismuth E., Sarda C. and Gadal P., Purification and characterization of chloroplast NADP-isocitrate dehydrogenase from mixotrophic tobacco cells, Comparison with the cytosolic isoenzyme, *Plant Physiology*, **105**, 593-600 **(1994)**

11. Giet C., Malate dehydrogenase isoenzymes: cellular location and role in the flow of metabolites between the cytoplasm and cell organelles, *Bioch. Biophy. Acta*, **1100**, 217-234 **(1992)**

12. Govindarajulu M., Pfeffer P.E., Abubaker J., Douds D.D., Allen J.W., Bucking H., Lammers P.J. and Shachar H.Y., Nitrogen transfer in the arbuscular mycorrhizal symbiosis, *Nature*, **435**, 819-823 **(2005)**

13. Hodges M., Flesch V., Gálvez S. and Bismuth E., Higher plant NADP+ -dependent isocitrate dehydrogenases, ammonium assimilation and NADPH production, *Plant Physiol. Bioch.*, **41**, 577-585 **(2003)**

14. Masclaux-Daubresse C., Vedele D.F., Dechorgnat J., Chardon F., Gaufichon L. and Suzuki A., Nitrogen uptake, assimilation and remobilization in plants: challenges for sustainable and productive agriculture, *Annals of Botany*, **105**, 1141-57 **(2010)**

15. Miflin B.J. and Habash D.Z., The role of glutamine synthetase and glutamate dehydrogenase in nitrogen assimilation and possibilities for improvement in the nitrogen utilization of crops, *J. Exp. Botany*, **53**, 979-987 **(2002)**

16. Miller R., Jastrow J. and Reinhardt D., External hyphal production of vesicular- arbuscular mycorrhizal fungi in pasture and tallgrass prairie communities, *Oecologia*, **103**, 17-23 **(1995)**

17. Miransari M., Arbuscular mycorrhizal fungi and nitrogen uptake, *Arch. Microb.*, **193**, 77-81 **(2011)**

18. Nakmee P.S., Techapinyawat S. and Ngamprasit S., Comparative potentials of native arbuscular mycorrhizal fungi to improve nutrient uptake and biomass of Sorghum bicolor Linn, *Agr. Nat. Res.*, **50**, 173-178 **(2016)**

19. Palomo J., Gallardo F., Suarez M.F. and Canovas F.M., Purification and characterization of NADP-linked isocitrate dehydrogenase from Scots pine. Evidence for different physiological roles of the enzyme in primary development, *Plant Physiology*, **118**, 617-626 **(1998)**

20. Saito M., Enzyme activities of the Internal Hyphae and Germinated Spores of an Arbuscular Mycorrhizal Fungus, Gigaspora margarita Becker & Hall, *New Phytologist*, **129**, 425-431 **(1994)**

21. Salisbury F.B. and Ross C.W., Lipids and other natural products, In Plant Physiology, CBS Publishers and Distributors, Delhi **(1986)**

22. Setién I., Fuertes-Mendizabal T., Gonzalez A., Aparicio-Tejo P.M., Gonzalez-Murua C., Gonzalez-Moro M.B. and Estavillo J.M., High irradiance improves ammonium tolerance in wheat plants by increasing N assimilation, *J. Plant Physiol.*, **170**, 758-771 **(2013)**

23. Shapiro B.M. and Stadtman E.R., Glutamine synthetase (Escherichia coli), *Methods in Enzymology*, **17**, 910-922 **(1970)**

24. Skopelitis D.S., Paranychianaki N.V., Paschalidis K.A., Pliakonis E.D., Delis I.D., Yakoumakis D.I., Kouvarakis A., Papadakis A.K., Stephanou E.G. and Roubelakis-Angelakis K.A., Abiotic stress generates ROS that signal expression of anionic glutamate dehydrogenases to form glutamate for proline synthesis in tobacco and grapevine, *The Plant Cell*, **18**, 2767-2781 **(2006)**

25. Smith S.E., John B.J., Smith F.A. and Nicholas D.J., Activity of glutamine synthetase and glutamate dehydrogenase in Trifolium subterraneum L. and Allium cepa L.: Effects of mycorrhizal infection and phosphate nutrition, *New Phytologist*, **99**, 211-227 **(1985)**

26. Taiz L. and Zeiger E., Plant physiology: Mineral nutrition, Redwood, The Benjamin Cummings Publishing Company **(1991)**

27. Wang Z.A., Li Q., Ge X.Y., Yang C.L., Luo X.L., Zhang A.H., Xiao J.L., Tian Y.C., Xia G.X. and Chen X.Y., The mitochondrial malate dehydrogenase 1 gene GhmMDH1 is involved in plant and root growth under phosphorus deficiency conditions in cotton, *Scientific Report*, **5**, 10343 **(2015)**.

(Received 19th November 2019, accepted 31st January 2020)