Effect of *Rhizophagus irregularis* on Growth of Saffron (*Crocus sativus* L.) in Eastern Morocco

Maria Rimani[1]*, Ibtissam Mzabri[2], Khadija Charif[2], Zoheir Chafik[3] and Ezzahra Kharmach[1]
(1) Laboratory Bioresources, Biotechnology, Ethnopharmacology and Health, Department of Biology, Faculty of Sciences, University of Mohammed First, Oujda, Morocco; (2) Laboratory for Improving Agricultural Production, Biotechnology and the Environment, Department of Biology, Faculty of Sciences, University of Mohammed First, Oujda, Morocco; (3) Institute of Agricultural Technician Zraib, Berkane, Morocco. *Email of corresponding author: rimaniimaria@gmail.com

Abstract

Rimani, M., I. Mzabri, K. Charif, Z. Chafik and E. Kharmach. 2022. Effect of *Rhizophagus irregularis* on Growth of Saffron (*Crocus sativus* L.) in Eastern Morocco. Arab Journal of Plant Protection, 40(2): 182-187. https://doi.org/10.22268/AJPP-040.2.182187

Arbuscular mycorrhizal fungi (AMF) form mutualistic relationships with plant roots and can act as bio-fertilizers. In the same perspective, a study was conducted to investigate the possibility of a possible constitutive association of the arbuscular mycorrhizal fungus *Rhizophagus irregularis* L. (previously named *Glomus intraradices*) with saffron. The trial was conducted in the field at the experimental station of the Faculty of Sciences of Oujda (Morocco) by applying 3 doses of *R. irregularis* inoculum: 2, 4 and 6 ml per corm (T1, T2 and T3 treatments). Six months after planting, morphological, biochemical and mycorrhization parameters were measured. The results showed that the inoculation of saffron roots by *R. irregularis* was successful, which induced a significant increase in the number of leaves, the weight of stigmas and the percentage of daughter corms with large diameter. Similarly, the total chlorophyll content was increased, the highest value was recorded for the T3 treatment in April (0.04 mg/g MF), with an increase of 25% compared to the control.

Keywords: Arbuscular mycorrhizal fungus (AMF), inoculum, root colonization, total chlorophyll content, *Rhizophagus irregularis*, saffron.

Introduction

Saffron (*Crocus sativus* L.) is a sterile triploid (x= 8; 2n= 3x= 24) perennial geophytic herb (Ghaffari, 1986), belonging to the family *Iridaceae*. The genus *Crocus* comprises about 80 species distributed mainly in the Mediterranean area and southeast Asia (Giorgi *et al.*, 2015).

Crocus sativus, poetically known as «Red Gold» because it represents the most expensive spice in the world (Mzabri *et al.*, 2019), its stigmas have been used since the ancient times in religious ceremonies, as a spice in culinary practices, as a colorant agent in the preparation of perfumes and cosmetics, and for medicinal purposes (Chevalier, 1926). Nowadays, these anti-cancer and anti-oxidant properties are the subject of several recent studies to highlight interest in its medical properties (Gresta *et al.*, 2008).

Morocco places a special attention to the development of saffron which is considered among the main products of Moroccan soil. In 2015, the saffron plantation in Morocco covered a surface area of around 1600 ha with an average yield of 3.5 t, making Morocco the fourth largest saffron producer in the world (Mzabri *et al.*, 2019).

Saffron cultivation is quite demanding in terms of manual labour, and is mainly planted in the Taliouine-Taznakht region. This situation is advantageous for this region as well as for Morocco, where saffron production is essentially based on family work (Lage & Cantrell, 2009).

More than 95% of terrestrial plants can live in symbiosis with fungi (Smith & Read, 1997). Symbiotic associations between mycorrhizal fungi and plant roots influence plant health, vigour, and productivity (Wang & Qui, 2006), and expressively improve the absorption of macronutrients and micronutrients from the soil, especially phosphorus, and allow plants to grow in areas with low mineral nutrients (Cozzolino, 2010). These properties are highly beneficial for the cultivation of saffron which grows in a wide range of soil types, including poor soils (Gresta *et al.*, 2008) and tolerate the ban on the use of chemical inputs for organically grown saffron, as is the case in Morocco.

The objective of the present study was to assess the possibility of a constitutive association between the mycorrhizal fungus and saffron corms and to investigate how this association supports the growth and development of saffron.

Materials and methods

Plant material and growing conditions

The product, which is associated with saffron roots, is a fungal preparation in the form of liquid inoculum based on spores of *R. irregularis* L. strain DAOM 197198 (synonym *Glomus irregulare*; formerly *Glomus intraradices*). It is a natural strain isolated from white ash (*Fraxinus americana*) in Quebec, with a content of 1000 spores/ml. The product was obtained by sporulation in an aseptic culture medium.

The trial was carried out in the field at the Experimental Station of the Faculty of Sciences of Oujda, at an altitude of 468 m, a latitude of 34' 39' 07' North and a longitude of 01 01 53' 01' West, with an arid to mild winter climate (Mzabri *et al.*, 2017). Nine pots of each treatment (3 inoculated groups and 1 uninoculated control group), with a

https://doi.org/10.22268/AJPP-040.2.182187

diameter and depth of 11 cm, were used to cultivate *C. sativus* seedlings. These plastic pots were filled with sterile soil consisting of a mixture of peat and sand (2:1 v: v). For the group inoculated directly at the root part, corms were subjected to three inoculum concentrations of *R. iregularsis* mycorrhizal solution: 2 ml/corm (T1), 4 ml (T2) and 6 ml (T3). Results were compared to a control (T0) that received only water.

Immediately after planting, a first application of 2 ml/corm of the mycorrhizal solution was applied for all the treatments (T1, T2 and T3), one month later, a second application of 2ml/corm was applied for the T2 and T3 treatments, and in the third month a contribution of 2ml/corm was applied only for the T3 treatment, to have at the end the T1 inoculated with 2 ml/corm, T2 inoculated with 4 ml/corm and T3 inoculated with 6 ml/corm. The applications were added directly to the rhizospheric zone.

The measured parameters

To detect the presence or absence of mycorrhizae, the root system of the inoculated plants was monitored after 6 months of inoculation. The leaves, corms, and roots were sampled separately, while randomly selected root parts of the plants were cleaned, especially the fine lateral roots, under a moderate jet of water before being stained according to the protocol of Phillips and Haymann (Vierheilig *et al.*, 2005). An optical microscope was used to observe the association between the *R. irregularis* and roots of *Crocus sativus* at x400 magnification after Trypan blue staining.

To evaluate the effect of mycorrhizae on saffron growth, morphological parameters such as the fresh and dry weight of the stigmas and the increase in leaf length were assessed. At the end of the growing cycle, after digging out the plants, the aerial part is separated from the underground part, the number and weight of the corms (using a precision balance) were measured. Corms were distinguished based on size: large (>2.5cm), medium (1.5cm <g<2.5cm) and small (<1.5cm). Total chlorophyll (TCT) was determined using the method of Tran *et al.* (1995).

Experimental design and statistical analyses

The experimental device adopted is the complete randomized block design (BAC), consisting of 3 blocks with a total of 36 tufts of saffron, (9 tufts/treatment x 4 treatments), where the blocks indicate replications, and the sub-blocks represent the treatments. The results were subjected to descriptive statistical analysis and analysis of variance (ANOVA) using SPSS software version 23. The comparison of the means was done by the Duncun multiple range test at P=0.05.

Results

Microscopic observation revealed that all inoculation treatments with AMF showed an association between the vesicular-arbuscular mycorrhizal fungus *R. irregularis* and the roots of *Crocus sativus* (Figure 1).

The results of the fresh and dry weight of the stigmas showed that the highest weight was recorded in both mycorrhizal treatments with the lowest doses T2 followed by T1. These values were 0.030 g; 0.028 g (fresh weight) and 0.012g; 0.011g (dry weight), for T2 and T1 treatments, respectively, compared with 0.024 g (fresh weight) and 0.009 g (dry weight) for T3 treatment and 0.02 g (fresh weight) and 0.008 g (dry weight) for the T0 control (Figure 2), and the differences compared with the control were significant (P<0.05). Likewise, AMF inoculum had a moderate effect on mean leaf length compared to the control, although the most significant increase were observed in the T2 treatment during the month of February (Figure 3).

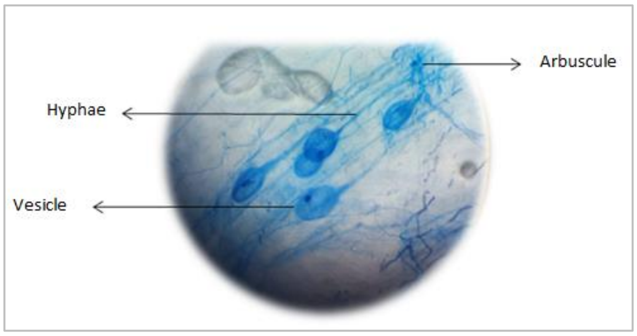

Figure 1. Staining of a mycorrhizal root of *C. sativus* and visualization of arbuscules, vesicles, and hyphae observed at 400 X magnification.

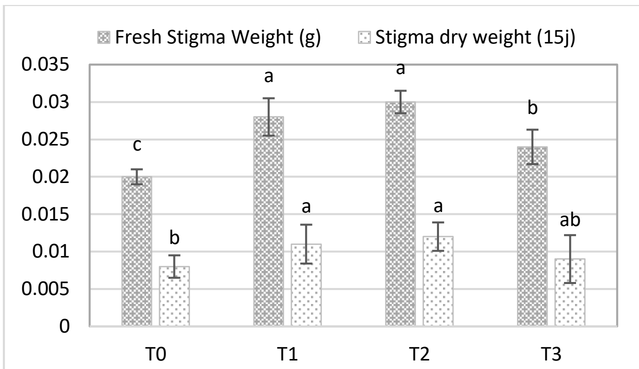

Figure 2. The effect of mycorrhizal treatments (T0= 0 ml; T1= 2 ml, T2= 4 ml and T3= 6 ml inoculum) on the fresh and dry weight of the stigma of the saffron (the results were the average of three replications).

The results in Figure 4 show that the percentage of diameter categories varied according to the mycorrhizal treatment applied. The T1 treatment showed a high fraction of the "large dimeter" category (>2.5 mm) compared to the other treatments, which was considered to be the most interesting from an agronomic point of view.

The results of the variation in total chlorophyll content (a+b), as shown in Figure 5, the AMF treatments showed a peak in December corresponding to 0.046; 0.028 and 0.058 mg/g MF for the three treatments of 2 ml, 4 ml and 6 ml inocula, respectively. Compared to the control, Chlorophyll content was generally not increased until February. This increase became significant in April when the chlorophyll content reached a maximum of 0.04 mg/g MF, and was 25% higher than the control. However, these variations are significant between the different mycorrhizal treatments compared to the control only during the months of March and April at P=0.05.

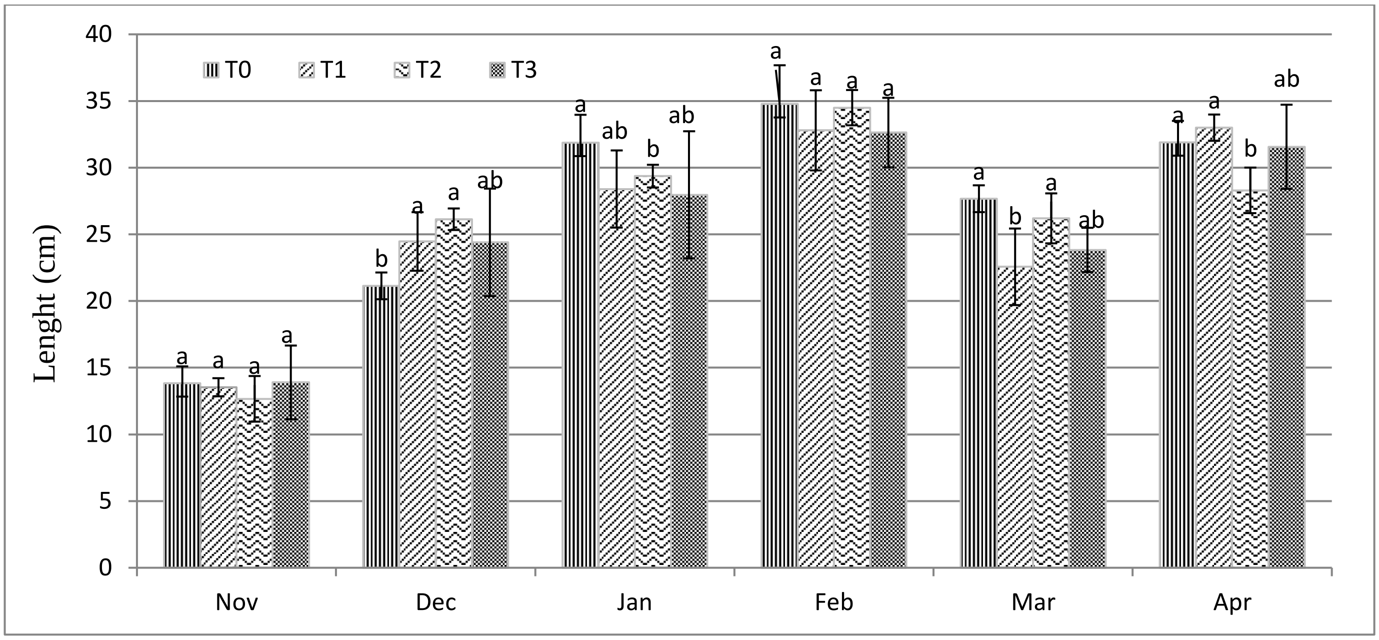

Figure 3. The effect of different treatments (T0= 0 ml; T1= 2 ml, T2= 4 ml and T3= 6 ml of inoculum) on average leaf length (results are the average of three replicates).

Discussion

Arbuscular mycorrhizal fungi (AMF) belong to the class Glomeromycetes (Garbaye, 2013; Gavériaux, 2012; Wang & Qui, 2006). Saffron bulbs produce fibrous and contractile roots (Kalesi *et al.*, 2004), which enable corms to form a symbiotic and natural association with soil fungi. The literature on naturally associated AMF with saffron in the Iranian-Turanian region is *Acaulospora morrowiae* and *Glomus coronatum* (Zare *et al.*, 2000). However, studies conducted at the national level (Morocco) on the interaction of soil fungi and saffron reveal that the species *Funneliformis* and *Rhizoglomus* were the most abundant (Chamkhi *et al.*, 2019). The AMF inoculations with *R. irregularis* used in this study successfully colonized *C. sativus* roots from each treatment and intercellular hyphal, interbuscular, and vesicular structures were present in all inoculated treatments. Inoculation with endomycorrhizal fungi significantly improved plant growth. Several studies indicate that soil symbiont has a potentially strong impact on growth and yield on several crops worldwide (Kianmehr, 1981; Lone *et al.*, 2015; Shuab *et al.*, 2014). Zare *et al.* (2000) indicated that the application of mycorrhizal inoculum in saffron fields could improve its yield and make possible the cultivation of saffron in more stressful habitats. In this study, inoculation even with low doses of 2 ml and 4 ml significantly improved the morphology of *C. sativus*, which is consistent with the results of other authors who have shown that the fresh and dry biomass of saffron was significantly increased in plants inoculated with AMF compared to those not inoculated (Kianmehr, 1981; Mohebi-Anabat *et al.*, 2015; Shuab *et al.*, 2014; Zhu *et al.*, 2014).

Contrary to what has been reported on the significant improvement in plant size and height in several species such as saffron (Mohebi-Anabat *et al.*, 2015), onion (Bolandnazar *et al.*, 2007), fonio (Ndoye *et al.*, 2016), and maize (Mier, 2015), no significant effect on height was found in this study.

The effect of mycorrhization on reserve organs was reflected by a significant improvement in corm diameter compared to the control; the same trend was reported by Charron *et al.* (2001), who found that application of a mycorrhizal fungus had a significant effect on the final diameter of the onion bulb. These results could be explained by the fact that the number of leaves increased and subsequently the quantity of reserves stored in the corms during the vegetative phase as a result of improved photosynthetic activity. McGimpsey *et al.* (1997) showed that large corms improve flowering density, and give larger son corms for the next season. For corm weight, Bolandnazar *et al.* (2007) and Ojala *et al.* (1983) found that mycorhized plants had a higher weight of onion bulbs than non-mycorrhized plants, which is the same in our study where *R. irregularis* showed an effect on saffron weight, although not significant.

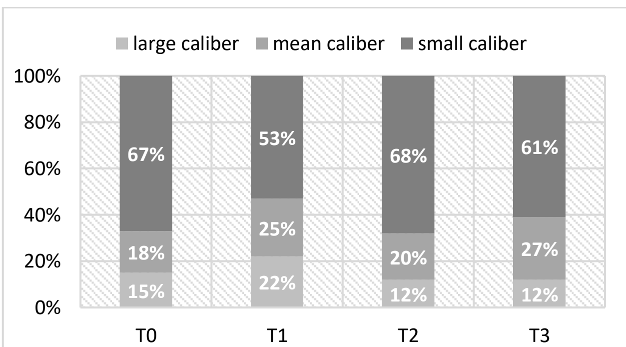

Figure 4. Effect of mycorrhizal treatments (T0= 0 ml; T1= 2 ml, T2= 4 ml, and T3= 6 ml inoculum) on saffron corm diameter. (The values represent the average of 3 replications).

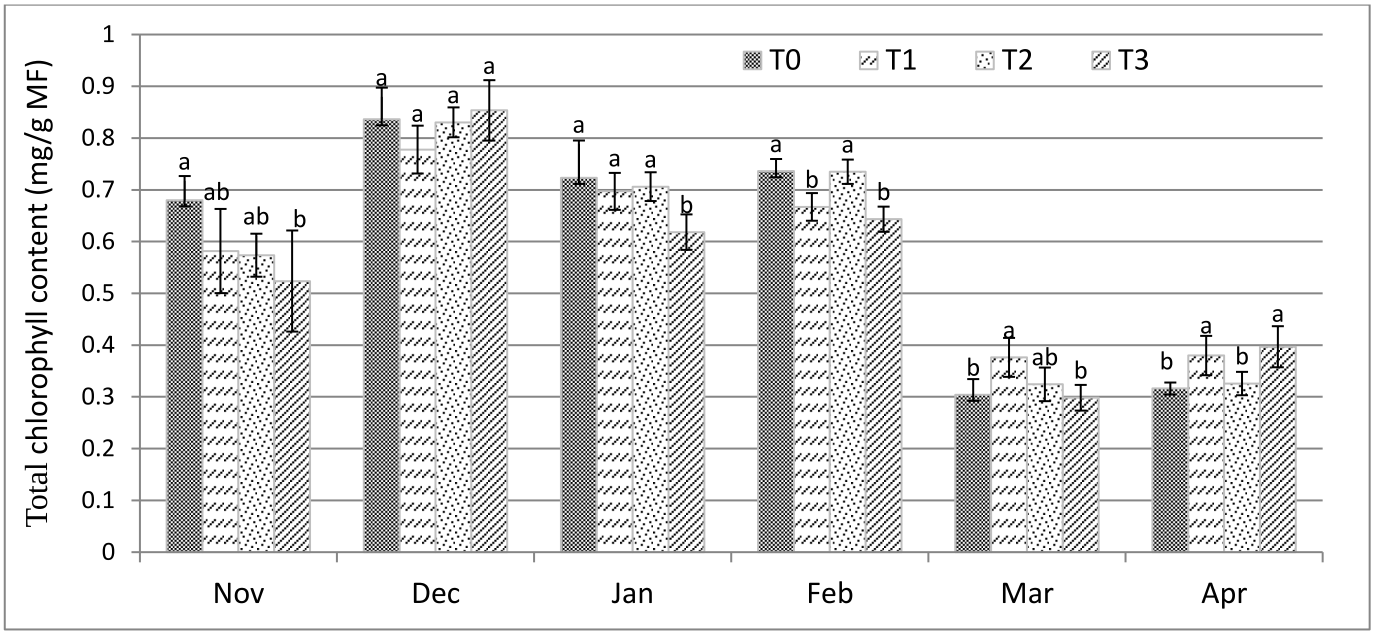

Figure 5. Effect of different treatment doses (0 ml; 2 ml, 4 ml, and 6 ml inoculum) on total chlorophyll content (a+b) (results are the average of three replicates).

Colonization by AMF increased the concentration of photosynthetic pigments compared to non-mycorrhized plants. The high amount of chlorophyll in inoculated plants increased the rate of photosynthesis, and subsequently the rates of photosynthetic storage and export (Haneef *et al.*, 2013).

The association of the vesicular-arbuscular mycorrhizal fungus *R. irregularis* L. with saffron was demonstrated in this study. Artificial inoculation with different doses of *Rhyzophagus irregularis* resulted in a net improvement in seedling growth and nutrition with superiority at the lowest dose (2 ml). These results encourage us to conduct a field study in the different saffron-growing regions to demonstrate both the high rate of saffron colonization by this fungus and its ability to adapt to edapho-climatic conditions.

الملخص

ريماني، ماريا، إبتسام مزابري، خديجة شريف، زهير شفيق والزهرة خرماش. 2022. تأثير الفطريات الجذرية في نمو نبات الزعفران في شرق المغرب. مجلة وقاية النبات العربية، 40(2): 182-187. https://doi.org/10.22268/AJPP-040.2.182187

تشكّل الفطريات الجذرية (الميكوريزا) (AMF) علاقاتٍ متبادلة مع جذور النباتات ويمكنها أن تعمل كأسمدةٍ حيوية. وفي المنظور نفسه، أجريت دراسة لاستقصاء إمكانية الارتباط التكويني المحتمل للفطر الجذري *Rhizophagus irregularis* مع نبات الزعفران. أجريت التجربة ميدانياً في محطة التجارب بكلية العلوم بوجدة (المغرب) بتطبيق ثلاثة تراكيز (2، 4 و 6 مل/بصيلة) من محلول الميكوريزا *Rhizophagus irregularis*. وبعد ستة أشهر من الزراعة، تمّ قياس المؤشرات الشكلية والكيميائية الحيوية المتعلقة بالميكوريزا. أظهرت النتائج نجاح تلقيح جذور الزعفران بالفطر *R. irregularis*، الأمر الذي أدّى لحدوث زيادةٍ كبيرة في عدد الأوراق وتحسين وزن المياسم، ونسبة البصيلات البنت المنبثقة بأقطار كبيرة. وعلى نحوٍ مشابه، فقد زاد محتواها من اليخضور الكلي، وتمّ الحصول على أفضل النتائج عند استعمال الجرعة 6 مل (T3) من لقاح الفطر الجذري، وسجّلت أعلى قيمة (0.04 مغ/غ من الوزن الرطب) في شهر نيسان/أبريل بزيادة قدرها 25% مقارنةً بالشاهد.

كلمات مفتاحية: الفطور الجذرية، ميكوريزا، لقاح، الارتباط التكويني الجذري، نسبة اليخضور الكلي، *Rhizophagus irregularis*، الزعفران.

عناوين الباحثين: ماريا ريماني[1]*، إبتسام مزابري[2]، خديجة شريف[2]، زهير شفيق[3] والزهرة خرماش[1]. (1) مختبر المصادر الحيوية والتكنولوجيا وعلم الأدوية والصحة، قسم الأحياء، كلية العلوم، جامعة محمد الأول، وجدة، المغرب؛ (2) مختبر تحسين الإنتاج والتكنولوجيا الحيوية والبيئية، جامعة محمد الأول، وجدة، المغرب؛ (3) معهد التقني الزراعي زريب، بركان، المغرب. *البريد الالكتروني للباحث المراسل: rimaniimaria@gmail.com

References

Bolandnazar, S.A., M.R Neyshabouri, N. Aliasgharzad and N. Chaparzadeh. 2007. Effects of Mycorrhizal Colonization on Growth Parameters of Onion under Different Irrigation and Soil Conditions. Pakistan Journal of Biological Sciences, 10: 1491-1495. https://doi.org/10.3923/pjbs.2007.1491.1495

Chamkhi, I., Y. Abbas, K. Tarmoun, J. Aurag and L. Sbabou. 2019. Morphological and molecular characterization of arbuscular mycorrhizal fungal communities inhabiting the roots and the soil of saffron (*Crocus sativus* L.) under different agricultural management practices. Archives of Agronomy and Soil Science, 65: 1035-1048. https://doi.org/10.1080/03650340.2018.1548012

Charron, G., V. Furlan, M. Bernier-Cardou and G. Doyon. 2001. Response of onion plants to arbuscular mycorrhizae: 2 Effects of nitrogen fertilization on biomass and bulb firmness. Mycorrhiza, 11: 145-150. https://doi.org/10.1007/s005720100122

Chevalier, A. 1926. La culture du Safran. Revue de botanique appliquée et d'agriculture coloniale, bulletin 59 : 407-419. https://doi.org/10.3406/jatba.1926.4426

Cozzolino, V., M. Pigna, V. Di Meo, A.G. Caporale and A. Violante. 2010. Effects of arbuscular mycorrhizal inoculation and phosphorus supply on the growth of *Lactuca sativa* L. and arsenic and phosphorus availability in an arsenic polluted soil undernon-sterile conditions. Applied Soil Ecology, 45: 262-268. https://doi.org/10.1016/j.apsoil.2010.05.001

Garbaye, J. 2013. La symbiose mycorhizienne, une association entre les plantes et les champignons, 1ère ed. 78026 Versailles cedex, Versailles, France. 280 pp.

Gavériaux, J.P. 2012. Les Glomeromycota - Mycorhizes VAM et *Geosophon pyriformis* (Kützing) Wettstein -. Bull. Soc. Mycol. Nord Fr, 92: 1-17.

Ghaffari, S.M. 1986. Cytogenetic studies of cultivated *Crocus sativus* (*Iridaceae*). J. Plant Systematics and Evolution, 153: 199-204. https://doi.org/10.1007/BF00983687

Giorgi, A., D. Bertoni, A. Manzo and S. Panseri. 2015. L'Oro Rosso delle Alpi. Manuale tecnico-scientifico di produzione dello zafferano, Biblion edizioni, Milano, Italy. 131pp.

Gresta, F., G.M. Lombardo, L. Siracusa and G. Ruberto. 2008. Saffron, an alternative crop for sustainable agricultural systems. Agronomy for Sustainable Development, 28: 95-112. https://doi.org/10.1051/agro:2007030

Haneef, I., S. Faizan, R. Perveen and S. Kausar. 2013. Role of arbuscular mycorrhizal fungi on growth and photosynthetic pigments in (*Coriandrum sativum* L.) grown under cadmium stress. World Journal of Agricultural Sciences, 9: 245-250. https://doi.org/10.5829/idosi.wjas.2013.9.3.1734

Kalesi, M., B. Sh. Behboodi and H. Ebrahimzadeh. 2004. Development and contraction of contractile roots in *Crocus sativus*. Acta Horticulturae, 650: 55-58. https://doi.org/10.17660/ActaHortic.2004.650.4

Kianmehr, H. 1981. Vesicular-arbuscular mycorrhizal spore population and infectivity of Saffron (*Crocus sativus*) in Iran. New Phytologist, 88: 79-82. https://doi.org/10.1111/j.1469-8137.1981.tb04570.x

Lage, M. and C.L. Cantrell. 2009. Quantification of saffron (*Crocus sativus* L.) metabolites crocins, picrocrocine and safranal for quality determination of the spice grown under different environmental Moroccan conditions. Scientia Horticulturae, 121: 366–373. https://doi.org/10.1016/J.SCIENTA.2009.02.017

Lone, R., R. Shuab, K.A. Wani, M.A. Ganaie, A.K. Tiwari and K.K. Koul. 2015. Mycorrhizal influence on metabolites, indigestible oligosaccharides, mineral nutrition and phytochemical constituents in onion (*Allium cepa* L.) plant. Scientia Horticulturae, 193: 55–61. https://doi.org/10.1016/j.scienta.2015.06.032

McGimpsey, J.A., M.H. Douglas and A.R. Wallace. 1997. Evaluation of saffron (*Crocus sativus* L.) production in New Zealand. New Zealand Journal of Crop and Horticultural Science, 25: 159-168. https://doi.org/10.1080/01140671.1997.9514002

Mier, M.N. 2015. Impact d'un champignon mycorhizien à arbuscules (*Rhizophagus irregularis* MUCL 41833) sur le prélèvement du phosphore chez le maïs (*Zea mays*) en condition de stress salin. Mémoire de fin d'études. Faculté de Bioingénieurs, Université catholique de Louvain, Belgique.

Mohebi-Anabat, M., H. Riahi, S. Zanganeh and E. Sadeghnezhad. 2015. Effects of arbuscular mycorrhizal inoculation on the growth, photosynthetic pigments and soluble sugar of *Crocus sativus* (saffron) in autoclaved soil. International Journal of Agronomy and Agricultural Research, 6: 296-304.

Mzabri, I., M. Addi and A. Berrichi. 2019. Traditional and Modern Uses of Saffron (*Crocus Sativus*). Cosmetics, 6: 1-11. https://doi.org/10.3390/cosmetics6040063

Mzabri, I., M. Legsayer, F. Aliyat, M. Maldani, N. Kouddane, A. Boukroute, I. Bekkouch and A. Berrichi. 2017. Effect of Drought Stress on the Growth and Development of Saffron (*Crocus Sativus* L.) in Eastern Morocco. Acta Horticulturae, 1184: 55-62. https://doi.org/10.17660/ActaHortic.2017.1184.8

Ndoye, F., A.G. Diedhiou, M. Gueye, D. Fall, A. Barnaud, O.Sy. Mame, K. Noba, D. Diouf and A. Kane. 2016. Réponse du fonio blanc (Digitaria exilis Stapf) à l'inoculation avec des champignons mycorhiziens à arbuscules en conditions semi-contrôlées. Journal of Applied Biosciences, 103: 9784–9799. https://doi.org/10.4314/jab.v103i1.1

Ojala, J.C., W.M. Jarrell, J.A. Menge and E.L.V. Johnson. 1983. Influence of Mycorrhizal Fungi on the Mineral Nutrition and Yield of Onion in Saline Soil. Agronomy, 75: 255-259. https://doi.org/10.2134/agronj1983.00021962007500020023x

Tran, H.T., N.P. Degtyareva, N.N. Koloteva, A. Sugino, H. Masumoto, D.A. Gordenin and M.A. Resnick. 1995. Replication slippage between distant short repeats in Saccharomyces cerevisiae depends on the direction of replication and the RAD50 and RAD52 genes. Journal of Molecular Cell Biology, 15: 5607-17. https://doi.org/10.1128/mcb.15.10.5607

Shuab, R., R. Lone, J. Naidu, V. Sharma, S. Imtiyaz and K.K. Koul. 2014. Benefits of inoculation of arbuscular mycorrhizal fungi on growth and development of onion (*Allium cepa*) plant. American-Eurasian Journal of Agricultural and Environmental Sciences, 14: 527-535. https://doi.org/10.5829/idosi.aejaes.2014.14.06.12347

Smith, S.E. and D.J. Read. 1997. Myccorhizal symbiosis. 2nd Ed. Academic Press Ltd, London, UK. 605 pp.

Vierheilig, H., P. Schweiger and M. Brundrett. 2005. An overview of methods for the detection and observation of arbuscular mycorrhizal fungi in roots. Physiologia Plantarum, 125: 393-404. https://doi.org/10.1111/j.1399-3054.2005.00564.x

Wang, B. and Y.L. Qui. 2006. Phylogenetic distribution and evolution of mycorrhizas in land plants. Mycorrhiza, 16: 299-363. https://doi.org/10.1007/s00572-005-0033-6

Zare, R., W. Gams and A. Culham. 2000. A revision of *Verticillium* sect. Prostrata. I. Phylogenetic studies using ITS sequences. Nova Hedwigia, 71: 465-480.

Zhu, X.Q., C.Y. Wang, H. Chen and M. Tang. 2014. Effects of arbuscular mycorrhizal fungi on photosynthesis, carbon content, and calorific value of black locust seedlings. Photosynthetica, 52: 247–252. https://doi.org/10.1007/s11099-014-0031-z

Received: March 4, 2021; Accepted: March 12, 2022

تاريخ الاستلام: 2021/3/4؛ تاريخ الموافقة على النشر: 2022/3/12