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

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Abstract

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 (Ghaifari, 1986), belonging to the family Iridaceae. The genus Crocus comprises about 80 species distributed mainly in the Mediterranean area and southeast Asia (Giorghi 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 coloanrt 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 (Coozolini, 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
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. irregularis* 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 Duncan 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.](image)

![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).](image)
**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 mycorrhized 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.
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 *Rhizophagus irregularis* L. with saffron was demonstrated in this study. Artificial inoculation with different doses of *Rhizophagus 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.

**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).

### الملخص


تكشف الفطريات الجذرية (الميكوريزيا) علاقات متداخلة مع جذور النباتات ويمكنها أن تعمل كأداة حيوية. وفي المظهر نفسه، أجريت دراسة لاستطالة إمكانية الارتباط التكوني المحتمل للفطريات الجذرية *(Rhizophagus irregularis)* لتفتيح النباتات (الساحل) بتطبيق ثلاثة تراكيز (2 و 4 و 6 مل/صبيحة) من محلول الميكوريزيا *(Rhizophagus irregularis)* والميكوريات.commentary: تتعلق الفطريات الجذرية بالساحل. أظهرت النتائج نجاح تفتيح جذور الزعفران بالفطريات، الأمر الذي أدى لزيادة في نسب النباتات وتحسين وزن المياسم، وسهولة البرمجة الممثلة بأضواء كبيرة. وعلى نحو ملحوظ، فقد زاد محتوى من الخضروات الكلي، وتم الحصول على أفضل النتائج عند استخدام الجرعة من 6 مل (T3) من تفتيح الفطريات الجذرية، وسجلت أعلى قيمة (0.04/ع) من الوزن الرطب (ف) في شهر نيسان/أبريل بزيادة قدرها 25% مقارنة بالشاهد.

كلمات مفتاحية: الفطريات الجذرية، الميكوريزيا، تفتيح، الارتباط التكوني الجذرية، نسبة الخضروات الكلي، الزعفران.


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