Investigation on Arbuscular Mycorrhizal Fungi (AMF) associated with *Crocus sativus* in Khorasan Razavi and Southern Khorasan provinces (north east of Iran)

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Abstract

Iran is the largest producer of saffron (*Crocus sativus*) in the world. More than 80% of higher plant species have a mutual relationship with mycorrhizal fungi, which enhances the plant growth and its productivity. With identification of native arbuscular mycorrhizal fungi and their application, it could be possible to expand saffron cultivated area and increase the performance of arable lands. In the present study, native AMF species associated with saffron roots in Khorasan Razavi and Southern Khorasan provinces (north east of Iran), and nine species of arbuscular mycorrhizal fungi, viz., *Claroideoglomus claroideum*, *C. etunicatum*, *Corymbiglomus tortuosum*, *Funneliformis caledonius*, *F. geosporum*, *F. mosseae*, *Paraglomus albidum*, *Rhizophagus aggregatus* and *R. manihotis* were identified which are all newly recorded for saffron mycoflora of Iran. *Rhizophagus manihotis* and *F. mosseae* were the most frequent species in all soil samples. Although, the maximum plants and fungal growth and root colonization usually take place in spring, but in case of saffron, results showed that, this happened in autumn which indicates, the fungus has adapted itself to host plant life cycle. On the other hand, correlation coefficient between spore population and root colonization was very low for Torbat specimens, which could be related to other factors e.g. environmental and geographical conditions.

Keywords: Glomeromycota, root colonization, saffron, spore population

بررسی قارچ‌های آربوسکولار میکوریز همزیست زعفران در استان‌های خراسان رضوی و خراسان جنوبی

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خلاصه

ایران بزرگ‌ترین تولید کننده زعفران (*Crocus sativus* L.) در جهان است. قارچ‌های آربوسکولار میکوریز با ۸۰٪ از نهاندانگان از جمله بیشتر گیاهان زراعی رابطه همزیستی دارند و باعث افزایش رشد و محصول‌دهی آن‌ها می‌شوند. شناسایی و در آینده به کارگیری این قارچ‌ها می‌تواند به افزایش تولید و نیز گسترش سطح زیر کشت این محصول استراتژیک کمک شایانی نماید. در این مطالعه، نه گونه قارچ آربوسکولار میکوریز همزیست با زعفران، جدا شده از مزارع استان‌های خراسان رضوی و خراسان جنوبی شامل: *Claroideoglomus claroideum*، *C. etunicatum*، *Corymbiglomus tortuosum*، *Funneliformis caledonius*، *F. geosporum*، *F. mosseae*، *Paraglomus albidum*، *Rhizophagus aggregatus* و *R. manihotis* شناسایی شدند که همگی برای میکوفلور زعفران در ایران جدید هستند. گونه‌های *F. mosseae* و *R. manihotis* بیشترین فراوانی را داشتند و در

همه خاک‌های نمونه‌برداری شده وجود داشتند. نتایج نشان داد که تعداد هاگ‌ها در فصل پاییز بیشتر از فصول بهار و تابستان است. اگرچه بیشترین رشد گیاهان و قارچ‌ها و نیز تلقیح ریشه‌ها اغلب در فصل بهار اتفاق می‌افتد، اما در مورد زعفران بیشترین میزان کلونیزاسیون در فصل پاییز رخ داد که نشان می‌دهد قارچ‌های آربوسکولار میکوریز همزیست با زعفران چرخه زندگی خود را با دوره رشدی گیاه میزبان خود تطبیق داده‌اند. پایین بودن میزان همبستگی بین تعداد هاگ‌ها و همزیستی در ریشه‌ها در نمونه‌های تربت نشان از دخالت عوامل دیگری همچون شرایط محیطی و جغرافیایی دارد.

واژه‌های کلیدی: ایران، جمعیت هاگ، کلونیزاسیون ریشه، گلومرومایکوتا

Introduction

Crocus sativus L. (Family: *Iridaceae*), is commercially cultivated for the production of the spice saffron (Fernández 2004). According to FAO statistics (2004), Iran is the record holder of saffron export in recent years by about 80% of total world production. Arbuscular mycorrhizal fungi are worldwide distributed soil fungi, forming symbiosis with most plant families. Their importance in natural and semi-natural ecosystems is commonly accepted and materialized by improved plant productivity and diversity as well as increased plant resistance against biotic and abiotic stresses (Smith & Read 2008). They are increasingly considered in agriculture, horticulture and forestry programs, as well as for environmental reclamation to increase crop yield and health and also to limit the application of agrochemicals (Jeffries *et al.* 2002, Johansson *et al.* 2004). In Iran, there are very few studies about saffron mycorrhiza (Kianmehr 1981, Zare & Nakhaei 2000) and it is needed to explore different aspects of mycorrhizal association of this economically important plant. In the present study, therefore, we had a survey about native AMF species associated with saffron in Khorasan Razavi and Southern Khorasan provinces (north east of Iran).

Materials and Methods

During October 2009 to September 2010, specimens were collected from Khorasan Razavi and Southern Khorasan provinces (north east of Iran), where main saffron fields are located. Five fields of every site including: Khalil-Abad and Torbat (in the range of 35-38 N and 57-60 E) and Ferdows (in the range of 36-43 N and 51-58 E) were selected. Each specimen contained whole roots and surrounding soil of saffron corm. At least five samples of each field were collected randomly and mixed together; and a bulk sample of one Kg was prepared for the next examinations. AMF spores were isolated by wet-sieving and decanting technique (Gerdmann & Nicolson 1963, Brundrett *et al.* 1996) followed by centrifugation (Tommerup & Kidbay 1979) and was counted according to Daft & Hograth (1983). To obtain fresh and healthy spore, pot culture for every specimen was established. The trap plant was corn (*Zea mays* L.) and soil samples were used as inoculums. Root segments were cleared and stained according to Philips & Hayman (1970) and RLC (Root Colonized Length) percent was calculated according to grid line-intersection method (Giovanetti & Mosse 1980).

Result and Discussion

Nine species of arbuscular mycorrhizal fungi were identified as below:

Claroideoglomus claroideum (N.C. Schenck & G.S. Sm.) C. Walker & A. Schüßler

C. etunicatum (W.N. Becker & Gerd.) C. Walker & A. Schüßler

Corymbiglomus tortuosum (N.C. Schenck & G.S. Sm.) Błaszk. & Chwat

Funneliformis caledonium (T.H. Nicolson & Gerd.) C. Walker & A. Schüßler

F. geosporum (T.H. Nicolson & Gerd.) C. Walker & A. Schüßler

F. mosseae (T.H. Nicolson & Gerd.) C. Walker & A. Schüßler

Paraglomus albidum (C. Walker & L.H. Rhodes) Oehl, G.A. Silva & Sieverd., in Oehl, Silva, Goto & Sieverding

Rhizophagus aggregatus (N.C. Schenck & G.S. Sm.) C. Walker

R. manihotis (R.H. Howeler, Sieverd. & N.C. Schenck) C. Walker & A. Schüßler (Fig. 1).

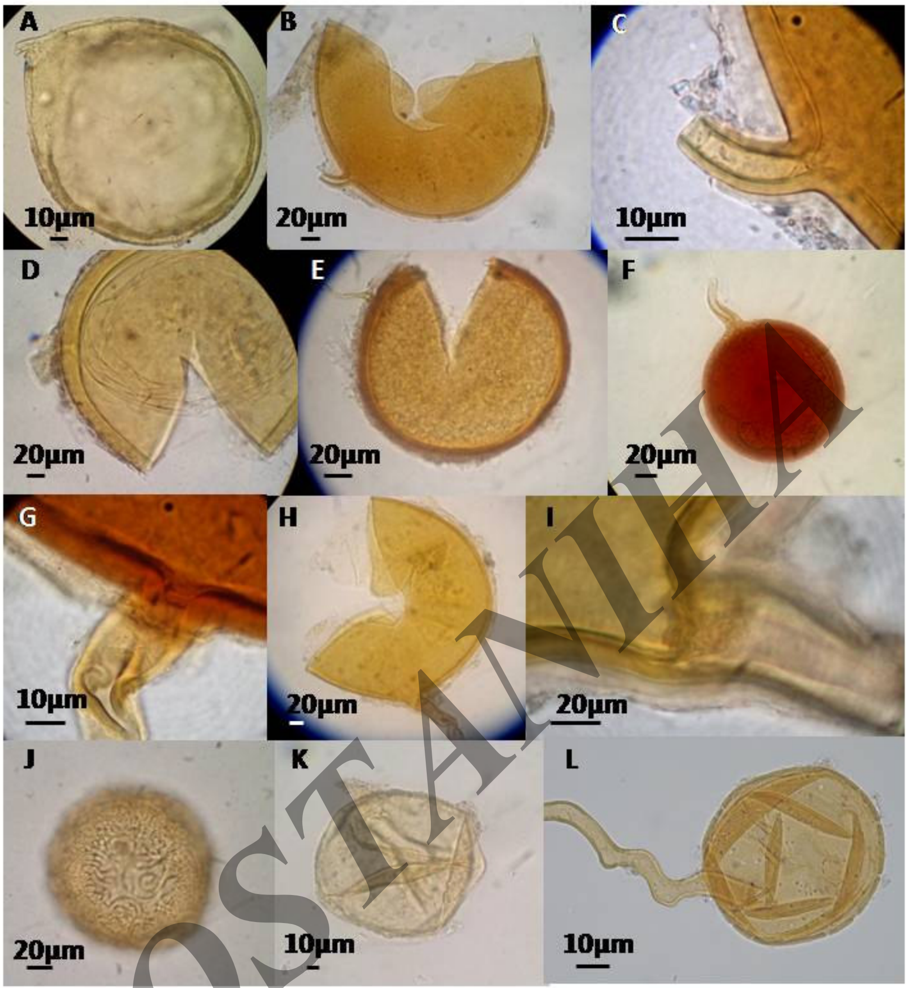

Fig. 1. A. *Paraglomus albidum*, B & C. *Funneliformis caledonium*, D. *Claroideoglomus claroideum*, E. *C. etunicatum*, F & G. *Funneliformis geosporum*, H & I. *Rhizophagus manihotis*, J. *Corymbiglomus tortuosum*, K. *Funneliformis mosseae*, L. *Rhizophagus aggregatus*.

All of these species are recorded for the first time from saffron rhizosphere from Iran of which, *Rhizophagus manihotis* and *Funneliformis mosseae* were the most frequent species in all areas (Fig. 2).

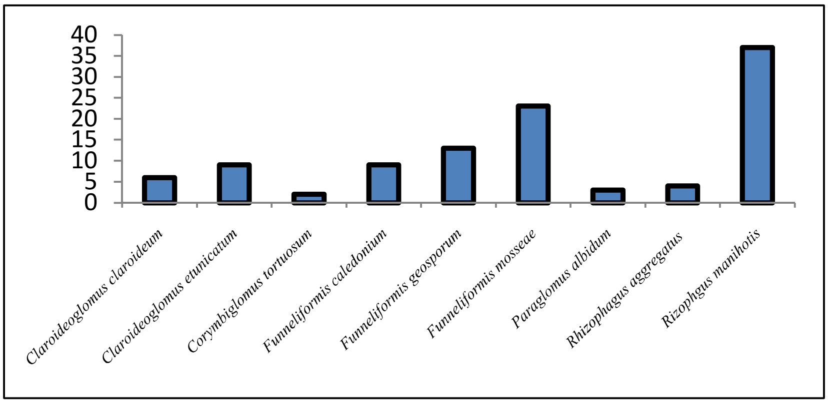

Fig. 2. Frequency of occurrence of AMF species in saffron rhizosphere.

Periodically sampled for enumerating number of spores in different sites indicated that, there is a variation in spore population in different intervals of the year. The result presented in Table 1, indicates that, the number of spores (1g of dry soil) and RLC% in Khalil-Abad, Torbat and Ferdows were 8–22, 25–34%; 6–19, 21–30% and 13–37, 35–41%, respectively. Correlation coefficient between spore populations and root colonization in the Khalil-Abad ($r^2 = 0.38$, $p < 0.05$), Ferdows ($r^2 = 0.74$, $p < 0.05$) and Torbat ($r^2 = 0.09$, $p < 0.05$) was calculated. The significant relationship between spore populations and root colonization in the sites Khalil-Abad and Ferdows was not found for Torbat site. Kianmehr (1981) also did not find any significant relationship between spore number of and root colonization in saffron. It seems, spore abundance is not a key factor for root colonization because, inoculums of arbuscular mycorrhizal fungi consist of different types of infective propagules, viz., spores, vesicles, hyphal fragments and hyphae from mycorrhizal root pieces (Brundrett 1991). Meanwhile, environmental conditions impact on the physiology of the plant host, soil chemical properties and physiological state of the fungal propagules. These complex interactions all influence infectivity and resultant mycorrhizal development. Therefore, in some cases, AMF spores require much more time for germination and some species of AMF are not able to germinate (McGee 1989) and in the natural ecosystem, most of the spores do not remain alive. Moreover, seasonal, environmental and geographical conditions act a crucial role in root colonization and AMF spore abundance, the circumstances which cannot be indexed in results. We also observed that, the number of spores in autumn was higher than spring and summer (Fig. 3).

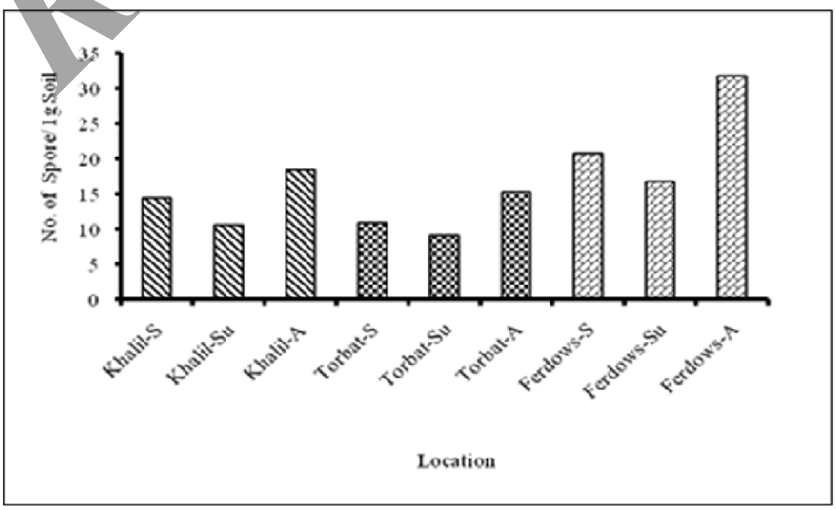

Fig. 3. Seasonal changes in spore population (S = Spring, Su = Summer and A = Autumn).

Spring is the growing season for most of the plants and maximum rate of root colonization occurs in this season, where in case of saffron, the maximum rate of root colonization observed in autumn. This indicates that, saffron AMF adapted its life cycle with the host plant. Effect of seasonal changes in the spore population in the rhizosphere of mycorrhizal plant has been reported by many researchers (Giovannetti 1985, Sylvia 1986). They showed that, spore abundance reaches to high level usually in mid- or late time of growth period. This could be related to reducing rate of carbohydrate content of plants in autumn, and making a stimulus to produce more spores for survival of AMF generation (Gupta *et al.* 2000). Klironomos *et al.* (1993) also reported that, the AMF spore frequency in the rhizosphere of maple tree (*Acer saccharum*) was higher in autumn than other seasons. It is, therefore, concluded that, according to the host plant, formation and function of AMF could be different both in the same habitat and the same season.

References

Brundrett, M.C. 1991. Mycorrhizas in Natural Ecosystems. Academic Press, pp. 171–313.

Brundrett, M., Bougher, N., Dell, B., Grove, T. & Malajczuk, N. 1996. Working with mycorrhizas in forestry and agriculture. ACIAR. Monograph series (No. 589.2 W6). Canberra: Australian Centre for International Agricultural Research, pp. 155–158.

Daft, M.J. & Hogarth, B.G. 1983. Competitive interactions amongst four species of *Glomus* on maize and onion. Transactions of the British Mycological Society 80(2): 339–345.

Fernández, J.A. & Pandalai, S.G. 2004. Biology, biotechnology and biomedicine of saffron. Recent Research Developments in Plant Science 2: 127–159.

Gerdemann, J.W. & Nicolson, T.H. 1963. Spores of mycorrhizal *Endogone* species extracted from soil by wet sieving and decanting. Transactions of the British Mycological Society 46(2): 235–244.

Giovannetti, M. & Mosse, B. 1980. An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. New Phytologist 84: 489–500.

Giovannetti, M. 1985. Seasonal variations of vesicular-arbuscular mycorrhizas and endogonaceous spores in a maritime sand dune. Transactions of the British Mycological Society 84(4): 679–684.

Gupta, M.L., Abdul Khaliq, R., Pandey, R. Shukla, S., Singh, H.N. & Kumar, S. 2000. Vesicular-arbuscular mycorrhizal fungi associated *Ocimum* spp. Journal of Herbs, Spices & Medicinal Plants 7(2): 57–64.

Jeffries, P., Craven-Griffiths, A., Barea, J.M., Levy, Y. & Dodd, J.C. 2002. Application of arbuscular mycorrhizal fungi in the re-vegetation of desertified Mediterranean ecosystems. Pp. 151–174. *In*: Gianinazzi, S., Schüepp, H., Barea, J.M. & Haselwandter, K. (eds). Mycorrhizal Technology in Agriculture. Birkhäuser Basel.

Johansson, J.F., Paul, L.R. & Finlay, R.D. 2004. Microbial interactions in the mycorrhizosphere and their significance for sustainable agriculture. FEMS Microbiology Ecology 48(1): 1–13.

Kianmehr, H. 1981. Vesicular-arbuscular mycorrhizal spore population and infectivity of saffron (*Crocus sativus*) in Iran. New Phytologist 88: 79–82.

Klironomos, J.N., Moutoglis, P., Kendrick, B. & Widden, P. 1993. A comparison of spatial heterogeneity of vesicular-arbuscular mycorrhizal fungi in two maple-forest soils. Canadian Journal of Botany 71(11): 1472–1480.

McGee, P.A. 1989. Variation in propagule numbers of vesicular-arbuscular mycorrhizal fungi in a semi-arid soil. Mycological Research 92(1): 28–33.

Phillips, J.M. & Hayman, D.S. 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Transactions of the British Mycological Society 55(1): 158-IN18.

Smith, S.E. & Read, D.J. 2008. Mycorrhizal Symbiosis, 3rd edn. Elsevier and Academic, New York, London, Burlington, San Diego.

Sylvia, D.M. 1986. Spatial and temporal distribution of vesicular-arbuscular mycorrhizal fungi associated with *Uniola paniculata* in Florida foredunes. Mycologia 28(5): 728–734.

Tommerup, I.C. & Kidby, D.K. 1979. Preservation of spores of vesicular-arbuscular endophytes by L-drying. Applied and Environmental Microbiology 37(5): 831–835.

Zare, M.H. & Nakhaei, A. 2000. Mycorrhizal symbiosis of saffron (*crocus sativus*) with two *Glominae* fungal species. Pazhuhesh-va-Sazandegi 13(3): 80–83.