The Journal of Microbiology, March 2004, p.60-63
Vol. 42, No. 1

NOTE

Use of Sucrose-Agar Globule with Root Exudates for Mass Production of Vesicular Arbuscular Mycorrhizal Fungi

Thangaswamy Selvaraj[1,2] and Hoon Kim[2,*]

[1]*Centre for Advanced Studies in Botany, University of Madras, Chennai 600-025, Tamil Nadu, INDIA,*
[2]*Department of Agricultural Chemistry, and Research Institute of Agricultural Sciences, Sunchon National University, Suncheon 540-742, Korea.*

(Received November 4, 2003 / Accepted March 2, 2004)

A sucrose-agar globule (SAG) was newly introduced to increase production of the vesicular arbuscular mycorrhizal (VAM) fungal spores, *Gigaspora gigantea* and *Glomus fasciculatum*. An SAG inoculum and a sucrose-agar globule with root exudates (SAGE) inoculum were prepared, and their spore productions were compared with a soil inoculum. When the SAGE was used as the inoculum on sucrose-agar medium plates the number of spores was increased (35% more than the soil inoculum). After the soil inoculum and SAGE were inoculated on an experimental plant, *Zingiber officinale*, the percentage root colonization, number of VAM spores, and dry matter content were analyzed. It was observed that the SAGE showed a higher percentage of root colonization (about 10% more), and increases in the number of spores (about 26%) and dry matter (more than 13%) for the two VAM fungal spores than the soil inoculum. The results of this study suggested that the SAGE inoculum may be useful for the mass production of VAM fungi and also for the large scale production of VAM fungal fertilizer.

Key words: VAM fungi, sucrose-agar globule inoculum, root exudates

The term vesicular arbuscular mycorrhizal (VAM) fungi define the fungi that form a special type of root, resulting from a mutual and symbiotic association between a plant and a fungus. VAM fungi play important roles in the increase of both the shoot weight and nutrient concentration in plants (Cooper, 1984). VAM fungi can efficiently and intensively extract soluble nutrients from soil, thus avoiding chemical fixation or leaching of solubilized or mineralized nutrients (Priya Rani *et al.*, 1999). VAM fungi are found in diverse habitats, from arctic to tropics, arid to semi-arid and stable plant communities to highly disturbed ecosystems (Bagyaraj *et al.*, 1979; Khan and Belik, 1995). They derive most of their essential organic matter from their symbiotic niche in roots, which in turn help better growth of their host plants due to enhanced phytochrome levels, the absorption of phosphorus and other mobile elements from soil, imparted tolerance to heavy metals and afford protection against disease, salinity and temperature extremes (Safir *et al.*, 1971; Benson and Covey, 1977; Gildon, 1981; Trappe, 1981; Tinker, 1984; Dueck *et al.*, 1986).

* To whom correspondence should be addressed.
(Tel) 82-61-750-3294; (Fax) 82-61-752-8011
(E-mail) hoon@sunchon.ac.kr

Even though VAM fungi can be a potent biofertilizer and nutrient remedifier, axenic cultures of VAM are currently far from being realized. The application of VAM fungi as a biofertilizer, from large scale production, is also a far off vision, as the significant development of complete a life cycle is only achieved in the presence of a host plant (Sahay *et al.*, 1998). Empirical tests of many chemical substances and physiological conditions have failed to provide sustained hyphal growth from germinated spores in the absence of host roots (Becard and Piche, 1992).

These failures led us to approach media preparations for the mass production of VAM fungi using substances favoring their growth. VAM fungi are able to germinate in the presence of sucrose and agar. Sucrose has the capacity to bind with agar, and when VAM spores were introduced they also germinated in the media. The sucrose-centrifugation method for VAM spore isolation (Smith and Skipper, 1979) has already proved that the VAM spores were not affected in the presence of sucrose, even up to 48% (w/v), whereas it helped to obtain more viable spores from the soil. When sucrose-agar with root-exudates (SAGE) was used for germination of VAM spores, there were more chances for successful germination, due to the presence of the root exudates. A trial study was performed by preparing a solid medium, sucrose-agar globule (SAG), for VAM

inoculum preparation, considering easy handling of the inoculum, viability of spores, long time storage and transportation of the inoculum. From this trial study, attempts were made to obtain the critical factors necessary to achieve successful growth of VAM fungi for mass production.

A few VAM fungal spores of *Gigaspora gigantea* and *Glomus fasciculatum* were isolated from the root-zone soils of some of the native herbaceous plant species by the methods of Gerdemann and Nicolson (Gerdemann and Nicolson, 1963). They were selected and identified by the synoptic keys of Gerdemann and Trappe (1974) and Schenck and Perez (1987). The spores were stored in microcentrifuge tubes, with Ringers solution (Danials and Graham, 1976), after disinfection with chloramine T (2%).

Root exudates

Root exudates were prepared from *in vitro*-grown seedlings of Maize (*Zea mays*). Maize seeds (no. 3245IR, Pioneer Hi-Bred International, USA) were sterilized with bleach and placed into 100 ml of 0.6% (w/v) agar in culture boxes, four seeds per box. After 2 weeks of growth at 25°C, the plants and their roots from 28 culture boxes were removed from the agar solution, and the solution centrifuged at 10,000 rpm for 30 min. Then the supernatant was sterilized by filtering and dried under vacuum. The resulting powder was resuspended in 100 ml of water and added to the sucrose-agar globule, without further dilution. Approximately 1 ml of the exudate corresponded to 1 g fresh root weight (Matvienko *et al.* 2001).

Sucrose-agar globule

Sucrose (250 g) and agar (250 g) were thoroughly mixed in a 1 liter beaker with a glass rod, making a 50% (w/v) mixture of each component. Sucrose was used as the binding agent for the agar as well as a non-toxic substance for the VAM fungal growth. Then the sucrose-agar mixture was shaped into small globular structures of 2 g in weight. Each globule had a hole in the center. Through the hole, the globule was injected with 10-20 VAM fungal spores and 1 ml of root exudates, and then the hole was sealed with sucrose-agar mixture. All these process were carried out under aseptic conditions. The behaviors of the fungal spores inside the globule were closely evaluated for hyphal growth and spore formation after inoculation onto plates containing sucrose-agar mixture. The soil inoculum was also prepared as a control, by the usual procedures, using onion as a host plant. The number of spores contained in 100 g of root-zone soil was analyzed (Raman and Mohankumar, 1988). The spores produced were counted from the soil inocula and the plates 10 weeks after inoculation.

The SAGE showed higher spore production than the soil and the sucrose-agar inocula without the root exudates (SAG) (Table 1). When the spores were analyzed, the soil inoculum showed 200 and 220 spores of *Gig. gigantea* and *Gl. fasciculatum*, respectively. The SAGE showed a higher number of spores, i.e. 35% more, 270 for *Gig. gigantea* and 297 for *Gl. fasciculatum*. The SAG showed only 180 and 195 spores of *Gig. gigantea* and *Gl. fasciculatum*, respectively.

Inoculation studies on Zingiber officinale

The SAGE containing VAM spores were inoculated to some ginger (*Zingiber officinale*) as the experimental

Table 1. Number of spores counted from soil inoculum and sucrose-agar globule inoculi[a]

Name of VAM spore	Number of spores		
	S	SAG	SAGE
Gigaspora gigantea	200	180	270
Glomus fasciculatum	220	195	297

[a]Soil inoculum was prepared from the host plant onion. After inoculation to the plant, the number of spores was counted from 100 g of the soil in 10 weeks. Sucrose-agar globule inoculi, SAG and SAGE, were prepared from sucrose-agar medium plates. After inoculation to the plates (20 g of globule per plate), the number of spores was counted from 100 g of the globules (five plates). S, soil inoculum; SAG, sucrose-agar globule inoculum without root exudates; SAGE, sucrose-agar globule inoculum with root exudates.

Table 2. Percentage[a] of root colonization, number of VAM spores and dry matter content in 10 week-old seedlings of *Zingiber officinale* treated with soil inoculum and sucrose-agar globule inoculum with root exudates containing *Gigaspora gigantea* or *Glomus fasciculatum*

	Type of inoculum	Root colonization (%)	VAM spores (per 100 g of soil)	Dry matter content (mg/plant)
Control		0	0	2,450±12.6
Gigaspora gigantea	S	80±1.6	320±1.4	3,755±13.5
	SAGE	90±1.4	410±1.2	4,315±12.3[a]
Glomus fasciculatum	S	87±1.5	376±1.3	4,125±12.4
	SAGE	93±1.3	468±1.8	4,540±12.6[a]

[a]Differences in the values are statistically significant (P=0.05). S, soil inoculum; SAGE, sucrose-agar globule inoculum with root exudates.

plants under potted conditions. Other ginger plants were also inoculated with soil inoculum, containing the same type of VAM spores, for a comparative analysis. For the inoculation with a soil-inoculum, the inoculum containing about 200 spores in 100 g of soil was used. Uninoculated plants were used as controls. The spores contained in 100 g of soil taken from the experimental plants 10 weeks after inoculation were counted from all the inoculated treatments. The dry matter contents of each inoculated treatment were also measured 10 weeks after inoculation.

The SAGE showed a higher percentage of root colonization, number of VAM spores and dry matter content than the soil inoculum treatments (Table 2). When *Gig. gigantea* was used in the SAGE, the 90% root colonization was higher (13% more) than the 80% for the soil inoculum. When *Gl. fasciculatum* was used in the SAGE, the 93% root colonization was also higher than the 87% for the soil inoculum (Table 2). When *Gig. gigantea* was used in the SAGE, the 410 VAM spores in root-zone soil was higher than the 320 with the soil inoculum. When *Gl. fasciculatum* was used in the SAGE, the 468 VAM spores were increased compared to the 376 with the soil inoculum (Table 2). The dry matter contents were also found to be higher in the SAGE treated with *Gig. gigantea* and *Gl. fasciculatum* compared to the value with the soil inoculum, at 4,315 and 3,755 mg/plant, and 4,540 and 4,125 mg/plant, respectively. In the control experiment, the dry matter content was least, at 2,450 mg/plant (Table 2).

The data obtained in this study clearly indicated that the growth of VAM fungi was not inhibited by the high concentration of sucrose, and showed that the sucrose concentration of 50% (w/v) in the sucrose-agar globule did not affect the growth of the VAM fungi and induced the growth of them when root-exudates were added. When treating the VAM spores on water-agar plates they were alive only for a short time after germination, *i.e.* up to a week. It was known that the formation of vesicles and spore structure were possible under identical conditions; however, these structures were not able to be maintained for a long time (Hepper, 1984; Becard and Piche, 1992). However, the mixture of 50% (w/v) of both sucrose and agar in the SAG improved the germination. The present result showed that the spore structures were formed and maintained alive for many days when kept at room temperature.

There was greater VAM spore formation in the SAGE compared with the soil inoculum. The increased number of spore production with the SAGE suggested that the inoculum was able to form more VAM spores in the presence of root exudates. Due to the absence of root exudates, the SAG inoculum showed less spores, while the soil inoculum showed a greater spore number because of the presence of host plant roots. It has been reported that many workers (Becard, 1994; Gulielmino *et al.*, 1994) have already germinated the spores of VAM fungi on water-agar plates in the presence of either root exudates or root pieces.

It was shown that the SAGE inoculation caused an increase in root colonization, greater spore production of VAM fungi and an elevated dry mass in the experimental plants. This may have been due to the presence of root exudates and their influence on maintaining the viability of spores in the globule, and a greater number of spores in the experimental plants, than in the soil inoculum. Earlier works (Hepper, 1984; Becard and Piche, 1992) revealed that tests with many chemical substances and physiological conditions failed to provide sustained hyphal growth from germinated spores in the absence of host roots. However, in the present investigation the SAGE was observed to be able to better maintain the growth of the hyphae and the development of VAM fungi spores and thus, is a better tool for the production of VAM fungi in a fertilizer form.

Acknowledgments

The provision of the facilities for the conduction of this study and the warm encouragement by the director of Centre for Advanced Studies in Botany, University of Madras, Chennai, India is gratefully acknowledged. This paper was supported in part by NON-DIRECTED RESEARCH FUND, Sunchon National University.

References

Bagyaraj, D.J., A. Manjunath, and I.R.B. Patil. 1979. Interaction between VA mycorrhiza and *Rhizobium* and their effect on soybean in field. *New Phytol.* 82, 141-145.

Becard, G. 1994. *In* J.M. Barea (ed.), Fourth European Symposium on Mycorrhizas, p. 131. IRNA Press, Spain.

Becard, G. and Y. Piche. 1992. *In* J.R. Norris, D.J. Read and A.K. Varma (eds.), Methods in microbiology, 24, 89. Academic Press, London.

Benson, N.R. and R.P. Covey. 1977. Response of apple seedlings to zinc fertilization and mycorrhizal inoculation. *Hort. Sci.* 11, 252-253.

Cooper, K.M. 1984. Physiology of VA mycorrhizal association. *In* Powell, C.L. and D.J. Bagyaraj (eds.), VA mycorrhiza, pp. 155-186. CRC Press, Boca Raton, Fla.

Danials, B.A. and S.O. Graham. 1976. Effects of nitrogen and soil extracts on germination of *Glomus mosseae* spores. *Mycologia* 68, 108-116.

Dueck, Th.A., P. Visser, W.H.O. Ernst, and H. Schat. 1986. Vesicular-arbuscular mycorrhizae decrease zinc-toxicity to grasses growing in zinc polluted soil. *Soil Biol. Biochem.* 18, 331-333.

Gerdemann, J.W. and T.H. Nicolson. 1963. Spores of mycorrhizal *Endogone* species extracted by wet sieving and decanting. *Trans. Brit. Mycol. Soc.* 46, 235-244.

Gerdemann, J.W. and J.M. Trappe. 1974. The Endogonaceae in the Pacific Northwest. *Mycologia Memoir.* No. 5, p 79.

Gildon, A. 1981. The influence of a vesicular-arbuscular fungus on the host plant uptake of heavy metals. Ph.D. Thesis, University

of London, UK.

Gulielmino, N., V. Bianciotto, I. Mortini, G. Lingua, and P. Bonafante. 1994. *In* J.M. Barea (ed.), Fourth European Symposium on Mycorrhizas, p. 149. IRNA press, Spain.

Hepper, C.M. 1984. *In* C.L Powell and D.J. Bagyaraj, (eds.), VA mycorrhiza, p. 95. CRC Press, Boca Ratoon.

Khan, A.G. and M. Belik. 1995. *In* A. Varma and B. Hock (eds.), Mycorrhiza-structure, functions, molecular biology and biotechnology, p. 627. Springer-Verlag, New York.

Matvienko, M., M.J. Torres, and J.I. Yoder. 2001. Transcriptional Responses in the Hemiparasitic plant *Triphysaria versicolor* to host plant signals. *Plant Physiol.* 127, 272-282.

Priya Rani, A. Agarwal, and R.S. Mehrotra. 1999. Growth responses in *Acacia nilotica* inoculated with VAM fungi (*Glomus mosseae*), *Rhizobium* sp. and *Trichoderma harzianum*. *Indian Phytopathol.* 52, 151-153.

Raman, N. and V. MohanKumar. 1988. Techniques in mycorrhizal research, University of Madras, Sivakami publications, Madras, p. 279.

Safir, G.R., J.S. Boyer, and J.W. Gerdemann. 1971. Mycorrhizal enhancement of water transport in soybean. *Science* 172, 581-583.

Sahay, N.S., Sudha, Archana Singh, and A. Varma. 1998. Trends in endomycorrhizal research. *Indian J. Experiment. Biol.* 36, 1069-1086.

Schenck, N.C. and Y. Perez. 1987. Manual for the identification of VA mycorrhizal fungi, p. 245. Univ. Florida, Gainsville.

Smith, W. and H.D. Skipper. 1979. Comparison of methods to extract spores of vesicular arbuscular mycorrhizal fungi. *Soil Sci. Soc. Amer. J.* 43, 722-725.

Tinker, P.B. 1984. The role of microorganisms in mediating and facilitating the uptake of plant nutrients from soil. *Plant Soil.* 76, 77-91.

Trappe, J.M. 1981. Mycorrhizae and productivity of arid and semi arid range lands. *In* J.T. Manassah and E.J. Brishey (eds.), Advances in food producing systems for arid and semi arid range lands, pp. 581-599. Academic Press, New York.