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COMMUNICATION II

Effect of Culture, Age and Pruning on Mass Production of the VA Mycorrhizal Fungus, *Glomus fasciculatum*.

ABSTRAK

Kultur spesis glomus fasciculatum *telah dibiakkan di atas rumput Rhodes* (Chloris gayana *Kunth) dengan menggunakan campuran perlit: tanah (1:1 mengikut isipadu) dalam pasu berukuran 16sm diameternya. Sebanyak 700 g substrat digunakan dalam setiap pasu, di mana penuaian telah dijalankan pada selang masa yang berlainan. Kultur yang dituai pada hari ke 75 selepas penanaman menghasilkan bilangan propagul yang maksimum. Dalam kajian kedua, didapati cantasan mengakibatkan bilangan propagul menurun sehingga minggu keempat. Walau bagaimanapun, jumlah propagul didapati meningkat semula selepas minggu ke 5 ke atas.*

ABSTRACT

Glomus fasciculatum *pot cultures were raised in Rhodes grass* (Chloris gayana *Kunth) using perlite – soilrite mix (1:1 by volume) in 16 cm pots holding 700 g substrate and were harvested at different intervals. Cultures harvested on the 75th day after sowing had the maximum number of infective propagules. In a second experiment, pruning was found to reduce the mycorrhizal propagules up to 4 weeks. However, the propagules increased from 5th week onwards.*

INTRODUCTION

Vesicular-arbuscular mycorrhizal (VAM) fungi, being obligate symbionts, are usually multiplied as 'pot cultures' using a suitable host (Wood, 1984). The inoculum usually consists of the root portion that is chopped and mixed with the potting medium (substrate) in which the plants are raised. Mycorrhizal colonization and sporulation is known to vary with plant age (Saif, 1977). There is no information available on the optimum age of the host for harvest of pot cultures raised for inoculum production. Perennial grasses are often used for maintenance and inoculum production of VAM fungi. These grasses require pruning. Pruning at an inopportune time (before the onset of sporulation) can affect inoculum production (Ferguson, 1981). Hence, the present study was undertaken to select the optimum age of host (Rhodes grass) for harvesting pot cultures of the VAM fungus, *Glomus fasciculatum* and to study the effect of a single pruning on the inoculum potential of the fungus.

MATERIALS AND METHODS

Earthen pots of 16 cm diameter were filled with 700 g of perlite-soilrite mix (1:1 by volume). Pots were inoculated with *G. fasciculatum* inoculum [originally obtained from University of California, Riverside @ 30 g/pot, having 2100 infective propagules/g as determined by MPN assay (Porter, 1979)] placed 2 cm below the substrate surface. Rhodes grass *(Chloris gayana* Kunth) seeds were sown and covered with a thin layer of the substrate. Sixteen pots with uniform plant populations (16 plants/pot) were maintained in a glass house with a mean temperature of 28–32°C and a maximum light intensity (PAR) of 820 ± 25 μEm^2s^{-1} and watered whenever necessary.

Eighty ppm N was given to the pot cultures as calcium ammonium nitrate in 2 split applications on days 10 and 30 after sowing, along with irrigation water. Ten ppm P, as rock phosphate was mixed uniformly with the substrate before sowing. Ruakura micronutrient solution (Smith

et al., 1983) with Zn, Cu and Mn at half strength was applied (50 ml/pot) on days 10, 25 and 40 after sowing. The host, substrate, forms and levels of nutrients used in this experiment were based on earlier trials to optimize mass multiplication of the VAM fungus *G. fasciculatum* (Sreenivasa, 1986). Four pots were harvested on days 45, 60, 75 and 90 after sowing.

Per cent root colonization was determined by staining the roots with trypan blue (Phillips and Hayman, 1970). Spores were separated from the substrate by wet sieving and decanting technique (Gerdemann and Nicolson, 1963), and inoculum potential was determined by MPN technique (Porter, 1979). Plant dry weights were recorded at each harvest.

In a second experiment pot cultures of *G. fasciculatum* were raised in 36 pots in a similar way as described previously. On day 75, plants in all the pots were uniformly pruned 1 cm above the surface of the substrate. Four pot cultures were sacrificed at weekly intervals from the day of pruning for 8 weeks to estimate per cent root colonization, spore count and inoculum potential following the techniques mentioned earlier.

RESULTS AND DISCUSSION

Harvesting the host 75 days after sowing resulted in the maximum number of mycorrhizal propagules. The percentage root colonization, extramatrical chlamydospore number and the number of infective propagules increased significantly up to 75 days of plant growth. The shoot and root dry weights also increased significantly only up to 75 days (Table 1). Perhaps the plants became

TABLE 1

Effect of plant age on per cent root colonization, extramatrical spore production and inoculum potential of *G. fasciculatum* inoculum, and shoot and root dry weights of Rhodes grass.

Plant age (days after sowing)	Per cent root colonization	Spore number/ 50 ml substrate	Number of infective propagules/g inoculum (x 10^7)	Shoot dry weight (g/plant)	Root dry weight (g/plant)
45	75 c	285 c	0.15	4.1 c	2.2 c
60	87 b	482 b	0.75	6.4 b	3.2 b
75	94 a	536 a	1.63	9.7 a	4.3 a
90	89 a	521 a	1.45	9.8 a	4.3 a

Root colonization values arcsin transformed before analysis. Non-transformed means presented.
Means with similar alphabets do not differ significantly at P = 0.05.

TABLE 2

Effect of pruning on per cent root colonization, spore number and inoculum potential of *G. fasciculatum* inoculum.

Weeks after pruning	Per cent root colonization.	Spore number/ 50 ml substrate	Number of infective propagules/g inoculum (x 10^7)
0	95 a	552 a	1.45
1	89 bc	509 bc	1.16
2	84 de	461 e	0.99
3	83 ef	421 gh	0.64
4	76 g	403 lh	0.47
5	82 ef	428 g	0.70
6	84 de	460 ef	0.84
7	88 cd	490 cd	0.95
8	91 b	511 b	1.11

Root colonization values arcsin transformed before analysis. Non-transformed means presented.
Means with similar alphabets do not differ significantly at P = 0.05.

pot bound after that. Colonization of the root by mycorrhizal fungi having a typical lag phase followed by a phase of rapid development and a constant phase has been reported earlier (Saif, 1977). In the present study with Rhodes grass *G. fasciculatum* combination grown in 16 cm pots, maximum number of infective propagules per g of the inoculum occurred on the 75th day. It is likely that more infective propagules could be produced over a longer period of time if larger pots are used.

Pruning significantly reduced per cent mycorrhizal root colonization, spore numbers and total infective propagules in the pot cultures of *G. fasciculatum*. The reduction continued up to 4 weeks after pruning. The number of mycorrhizal propagules started building up from the 5th week onwards. However, the mycorrhizal development 8 weeks after pruning was less than the level on the day of pruning (Table 2). Curtailment of translocated carbohydrate (photosynthate) supply to the roots after pruning affects the fungal symbiont considerably (Redhead, 1975). It is evident from the present study that when pot cultures are pruned, it is necessary to keep them for a period of slightly more than 8 weeks (perhaps 75 days) before harvesting them as mycorrhizal inoculum.

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REFERENCES

GERDEMANN, J.W. and T.H. NICOLSON (1963). Spores of mycorrhizal endogone species extracted from soil by wet sieving and decanting. *Trans. Br. Mycol. Sco.*, **46**: 235–244.

FERGUSON, J.J. (1981): Inoculum production and field application of vesicular-arbuscular mycorrhizal fungi. Ph.D. thesis, University of California, Riverside, USA.

PHILLIPS, J.M. and D.S. HAYMAN (1970): Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans. Br. Mycol. Soc.* **55**: 158–161.

PORTER, W.M. (1979): The 'most probable number method' for enumerating infective propagules of vesicular-arbuscular mycorrhizal fungi in soil. *Aust. J. Soil. Res.*, **17**: 515–519.

REDHEAD, J.F. (1975): Endotrophic mycorrhizas in Nigeria: Some aspects of the endotrophic mycorrhizal association of *Khaya grandifolia C.D.C.*: In Endomycorrhizas F.E. Sanoers, B. Mosse and P.B. Tinker, (eds). Academic Press, London. 447–459.

SAIF, S.R. (1977): The influence of stage of host development on vesicular-arbuscular mycorrhizae and endogonaceous spore population in the field grown vegetable crops. I. Summer grown crops. *New Phytol.*, **79**: 341–348.

SMITH, G.S., G.M. JOHNSTON and I.S. CORNFORTH (1983): Comparison of nutrient solutions for growth of plants in sand cultures. *New Phytol.* **94**: 537–548.

SREENIVASA, M.N. (1986): Inoculum production of the vesicular-arbuscular mycorrhizal fungus, *Glomus fasciculatum*. Ph.D. Thesis, University of Agricultural Sciences, Bangalore, India.

WOOD, T. (1984): Commercial pot culture inoculum production. Quality control and other headaches. In. *Proc. 6th North Amer. Con. Mycorrhizae.* R. Molina (ed.) Forest Res. Lab. Publ., Oregon, USA Press. P. 84.

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