ing a '*Neurospora* farm' for the extraction of industrially useful enzymes (invertase, cellulases, proteases, etc.). This is the real field model of a solid-state fermentation, which could yield valuable products. Such natural or man-made blooms could be of great value for future research.

1. Shear, C. L. and Dodge, B. O., *J. Agric. Res.*, 1927, **34**, 1019–1042.
2. Pandit, A. and Maheswari, R., *J. Biosci.*, 1996, **21**, 57–59.
3. Beadle, G. W. and Tautum, E. L., *Proc. Natl. Acad. Sci. USA*, 1941, **27**, 499–506.
4. Perkins, D. D., *Genetics*, 1992, **130**, 687–701.
5. Galagan, J. E. *et al.*, *Nature*, 2003, **422**, 859–868.
6. Turner, B. C., Raju, N. B. and Perkins, D. D., *Genome*, 1988, **30**, 298.
7. Turner, B. C., Perkins, D. D. and Fairfield, A., *Fungal Genet. Biol.*, 2001, **32**, 67–92.
8. Perkins, D. D., Turner, B. C. and Barry, E. G., *Evolution*, 1976, **30**, 281–313.
9. Shaw, D. E. and Robertson, D. F., *Mycology*, 1998, **12**, 154–158.

ACKNOWLEDGEMENTS. We acknowledge K.C.P. Sugar and Industries Corporation Ltd, Vuyyuru, Prof. Ramesh Maheshwari for helpful discussions and encouragement, Dr Kevin McKluskey, Fungal Genetics Stock Center, Kansas City, USA, for identification, Dr David D. Perkins, Stanford University, USA for reading the manuscript and the University Grants Commission (UGC-SAP-DRS-1) for financial assistance.

Received 21 June 2003; revised accepted 16 September 2003

K. RASHMI[†]
J. NAVEENA LAVANYA LATHA[†]
T. NAGA SOWJANYA[†]
P. KIRANMAYI[†]
M. VENUGOPAL RAO[#]
C. P. S. MENON[#]
P. MARUTHI MOHAN[†,*]

[†]*Department of Biochemistry, Osmania University, Hyderabad 500 007, India*
[#]*K.C.P. Sugar and Industries Corporation Ltd, Vuyyuru 521 165, India*
**For correspondence.*
*e-mail: maruthip@hotmail.com*

# Colonization of cruciferous plants by *Piriformospora indica*

This correspondence provides evidence for a positive interaction of *Piriformospora indica*, a symbiotic fungus with several members of Cruciferae. The evidence is based on *in vitro* and *in vivo* studies. *P. indica* was characterized by Verma *et al.*[1] and subsequently reported for its various positive relations with plants and also being opted as a bio-fertilizer, -protector, -regulator, -fungicide and -pesticide[2–7]. This fungus also serves as an agent for biological hardening of tissue-culture-raised plants[8–10]. Interaction with the terrestrial orchids was also demonstrated[10–14]. 18s rDNA and 28s-rDNA analysis assigned this fungus to Basidiomycetes. *P. indica* is cultivable on several defined synthetic media[15,16] and can colonize the root of a large number of hosts. Earlier, we have reported a non-interaction of the fungus with myc$^-$ mutants of soyabean (*Glycine max*) and pea (*Pisum sativum*)[10].

Seeds of *Brassica oleracea* var. *capitata* (cabbage), *Spinacia oleracea* (spinach) and *Brassica juncea* (mustard) were surface-sterilized with 0.02% $HgCl_2$ and placed on 0.7% water agar petri plates for germination in the dark. After 7–10 days when the plumule and radicle appeared, they were transferred to pre-fungus inoculated agar slants in glass tubes containing 1/10 concentration of MMN medium[17]. Plants alone (control) or in co-culture with fungus were allowed to grow for 60 days in the tissue-culture laboratory maintaining 16 h photoperiod, 1000 lux at 25 ± 2°C. Root infections were checked after 45 days. Root colonization was evaluated by grid intersect method followed by staining about 100 roots segments (1 cm each) with Trypan blue or Chlorozal black E. Root per cent colonization was calculated using the standard formula[18].

Figure 1 *a* shows better cabbage growth as a result of interaction with *P. indica*. An overall enhancement of plant biomass was recorded when the fungus interacted with other members like *S. oleracea*, *B. oleracea* var. *capitata* and *B. juncea* (Table 1). Chlamydospores were produced on the root surface and scattered away from the root, extramatrically. Profuse root colonization by the fungus was observed at inter- and intracellular levels and extramatrically (Figure 1 *b*, left). Figure 1 *b* (right) shows an enlarged view of the chlamydospores (arrows). At no stage did fungal hyphae invade the stellar tissue and aerial portions. An identical positive interaction was also recorded for *S. oleracea*, *B. oleracea* and *B. juncea*.

This observation was repeated in a glasshouse using sterile soil : sand mixture as substratum (3 : 1). The substratum was sterilized and filled in pre-washed and sterile earthenware pots (1 kg). The fungus was allowed to grow on Kaefer broth medium under constant shaking condition at 25 ± 2°C for 7 days (Figure 2 *a*, top). Small and large colonies giving an appearance of 'corals' (arrows) were washed to remove the adhering chemicals. Inoculum (1% w/v) was thoroughly mixed with soil/sand mixture. Pre-germinated sterile seedlings (four in number) were placed in each pot. Efforts were made to keep the root system in direct contact with the fungal inoculum[19]. Plants were grown in an environmentally controlled greenhouse maintained at 25 ± 2°C, 16 h light/8 h dark with fluorescent light intensity 1000 lux and relative humidity 70%. Plants were fertilized on alternate weeks with 1/10 diluted Hoagland solution[20] and irrigated with autoclaved tap-water on alternate days to maintain about 70% soil moisture. Plants were photographed after 45 days. Treated plants were superior in growth (Figure 2 *b*, right) leading to early flowering (see close arrows) and fruiting (open arrows). Roots were heavily colonized and produced a large number of chlamydospores as recorded for *in vitro* conditions.

Results demonstrate that this fungus colonizes a large number of photosymbionts of importance in agriculture, horticulture and forestry. It is capable of interacting and also promoting the overall development of members of Cruciferae,

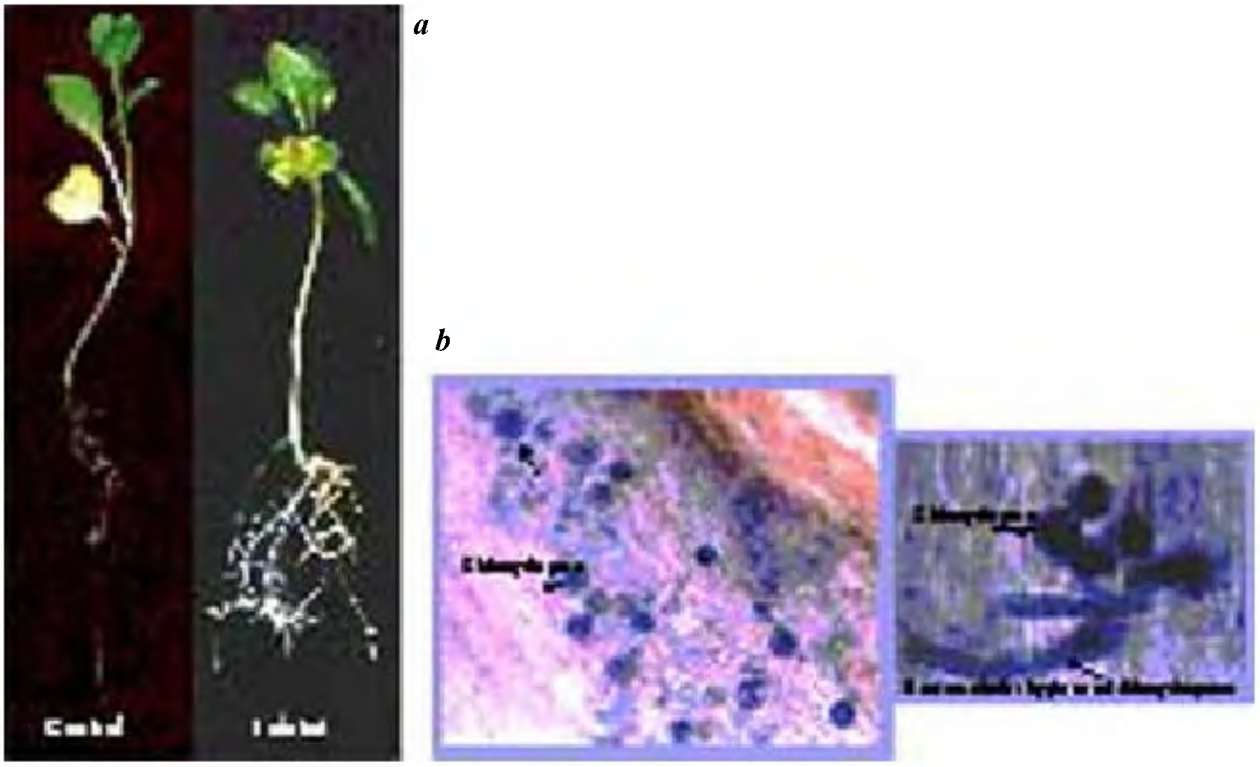


**Figure 1 *a–b*.** Interaction of *P. indica* with *B. oleracea* var. *capitata*.

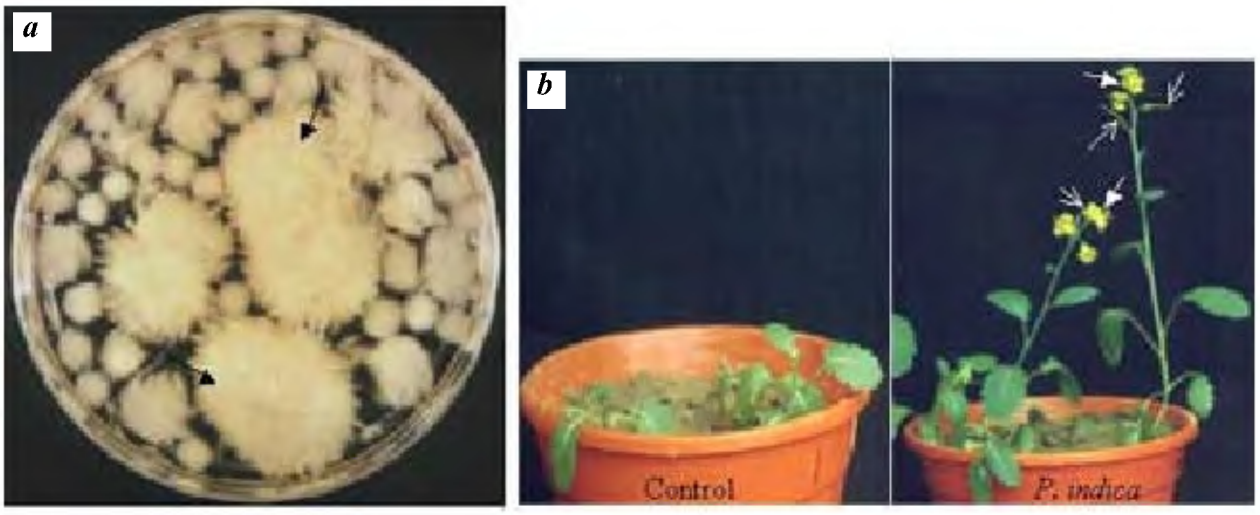


**Figure 2.** ***a***, *P. indica* biomass grown on Kaefer broth. ***b***, *In vivo* interaction of *P. indica* with *B. juncea*.

**Table 1.** Growth of photobionts after interaction with *P. indica*

| | Control | | | | Treated | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Photobionts | Fr. wt (g) | TL (cm) | SL (cm) | RL (cm) | Fr. wt (g) | TL (cm) | SL (cm) | RL (cm) | % Coln |
| Mustard | 0.10 ± 0.02 | 7.11 ± 1.84 | 3.18 ± 1.65 | 3.93 ± 1.12 | 0.12 ± 0.05 | 9.16 ± 6.31 | 4.60 ± 1.63 | 4.56 ± 5.85 | 50 |
| Cabbage | 0.13 ± 0.03 | 8.90 ± 3.02 | 4.50 ± 0.82 | 4.40 ± 3.01 | 0.18 ± 0.07 | 12.90 ± 2.48 | 7.10 ± 1.28 | 4.40 ± 3.01 | 70 |
| Spinach | 0.06 ± 0.03 | 11.6 ± 2.70 | 40.2 ± 1.52 | 7.40 ± 1.29 | 0.13 ± 0.03 | 13.20 ± 7.62 | 7.60 ± 4.27 | 5.60 ± 3.36 | 35 |

Data are average of five independent replications. Fr. wt, Fresh weight; TL, Total length (total height of plants); SL, Shoot length; RL, Root length; % Coln, Percentage colonization.

which are considered conventionally non-hosts for arbuscular mycorrhizal fungi (AMF) which interact with almost 90% of the terrestrial plants[21–23]. However, only limited members of the plant community have failed to interact; these belong to the families of Amaranthaceae, Chenopodiaceae, Cyperaceae Juncaceae, Proteaceae or with lupines and Cruciferae, etc.[22–26]. A careful perusal of the literature indicates that this statement may not be completely true[27,28]. Denison *et al.*[26] have emphasized that model systems are also important as a new research tool to understand the cooperation between microbes and the plants. Members of these families, including the model plant *Arabidopsis thaliana* lack symbiotic interactions such as mycorrhizae and rhizobia. The present study emphasized an interaction of endosymbiotic fungus *P. indica* with most members of cruciferae tested including *A. thaliana*[7]. This study further strengthens earlier results[18] that *P. indica* interacts with most plant groups and does not discriminate the members of Cruciferae, but fails to interact with myc$^-$ mutants (these also failed to interact with AMF)[10].

This observation opens up an approach for application of plant-promoting symbiotic fungus *P. indica* for better production of crops of agricultural and horticultural importance. It also provides models to understand the interaction between plants and plant-microbes at the molecular level.


1. Verma, S. *et al.*, *Mycologia*, 1998, **90**, 895–909.
2. Rai, M., Acharya, D., Singh, A. and Varma, A., *Mycorrhiza*, 2001, 123–128.
3. Singh, A., Sharma, J., Rexer, K. H. and Varma, A., *Curr. Sci.*, 2000, **79**, 101–106.
4. Singh, Ar., Singh, An. and Varma, A., *Indian J. Biotechnol.*, 2002, **1**, 372–376.
5. Singh, An., Singh, Ar., Kumari, M., Rai, M. K. and Varma, A., *Indian J. Biotechnol.*, 2003, **2**, 65–75.
6. Singh, An., Singh, Ar., Kumari, M., Kumar, S., Rai, M. K., Sharma, A. P. and Varma, A., *Biotechnology in Sustainable Biodiversity and Food Security* (ed. Prasad, B. N.), Science Publishers Inc., 2003, pp. 101–124.
7. Pham, G. H. *et al.*, *Plant Surface Microbiology* (eds Varma, A. *et al.*), Springer-Verlag, Germany, 2003, in press.
8. Sahay, N. S. and Varma, A., *FEMS Microbiol. Lett.*, 1999, **181**, 297–302.
9. Sahay, N. S. and Varma, A., *Curr. Sci.*, 2000, **78**, 126–129.
10. Varma, A. *et al.*, *Mycota IX Series* (ed. Hock, B.), Springer-Verlag, Berlin, 2001, pp. 123–150.
11. Blechert, O., Kost, G., Hassel, A., Rexer, R. H. and Varma, A., *Mycorrhizae* (eds Varma, A. and Hock, B.), Springer-Verlag, Germany, 1999, vol. 2, pp. 683–688.
12. Singh, A. and Varma, A., *Mycorrhizal Fungi* (ed. Mukherji, K. G.), Kluwer Academic Press, Amsterdam, 2000, pp. 265–288.
13. Varma, A., Verma, S., Sudha, Sahay, N., Britta, B. and Franken, P., *Appl. Environ. Microbiol. USA*, 1999, **65**, 2741–2744.
14. Singh, An., Singh, A., Rexer, K. H., Kost, G. and Varma, A., *J. Orchid*, 2001, **15**, 89–102.
15. Kaefer, E., *Adv. Genet.*, 1977, **19**, 33–131.
16. Pham, G. H. *et al.*, *Plant Surface Microbiology* (eds Varma, A. *et al.*), Springer-Verlag, Germany, 2003, in press.
17. Herrmann, S., Munch, J. C. and Buscot, F., *New Phytol.*, 1998, **138**, 203–212.
18. Giovannetti, M. and Mosse, B., *New Phytol.*, 1980, **84**, 489–500.
19. Varma, A. and Schuepp, H., *Crit. Rev. Biotechnol.*, 1995, **15**, 313–328.
20. Hoagland, D. R. and Arnon, D. I., *Univ. Calif., Agric. Exp. Stn. Circ.*, 1938, 346.
21. Bagyaraj, D. J. and Varma, A., *Advances in Microbial Ecology*, Plenum Press, England, 1995, vol. 14, pp. 119–142.
22. Smith, S. E. and Read, D. J., *Mycorrhizal Symbiosis*, Academic Press, London, 1997, p. 605.
23. Varma, A., *Mycorrhiza* (eds Varma, A. and Hock, B.), Springer-Verlag, Germany, 1999, pp. 521–556.
24. Read, D. J., *Mycorrhiza* (eds Varma, A. and Hock, B.), Springer-Verlag, Germany, 1999, pp. 3–34.
25. Varma, A. (ed.), *Mycorrhiza*, Springer-Verlag, Heidelberg, 1998, pp. 1–24.
26. Denison, R. D., Bledsoe, C., Kahn, M., Gara, F. O., Simms, E. L. and Thomashow, L. S., *Ecology*, 2003, **84**, 838–845.
27. Tester, M., Smith, S. E. and Smith, F. A., *Can. J. Bot.*, 1987, **65**, 419–431.
28. Leake, J. R., *New Phytol.*, 1994, **121**, 171–216.


ACKNOWLEDGEMENTS. We thank DBT, DST, UGC, CSIR and Dr Vivek Singhal, Biotech International Ltd for partial financial assistance.

Received 23 May 2003; revised accepted 15 September 2003

RINA KUMARI[†]
HARI KISHAN[†]
Y. K. BHOON[#]
AJIT VARMA[†,*]

[†]*School of Life-Sciences,*
*Jawaharlal Nehru University,*
*New Delhi 110 067, India*
[#]*Sri Venkateswara College,*
*University of Delhi,*
*Dhaula Kuan,*
*New Delhi 110 021, India*
**For correspondence.*
*e-mail: ajitvarma73@hotmail.com*

# Larval case renovation – a unique behaviour in bagworm moth, *Eumeta crameri* Westwood

Bagworms are a group of highly specialized lepidopterans belonging to the family Psychidae and exhibit extreme development of sexual dimorphism. Males are winged whereas females lack functional appendages. Larvae of both males and females, soon after hatching from the eggs climb up to the top of their host plants in order to have an access to the soft and palatable tips of the growing shoots. They construct a small but tough bag of silk of either cylindrical or conical shape and glue small fragments of plant tissues around their cases. Larvae always keep their body inside the cases. While moving about, their head and thorax are protruded out so that they move forward on their thoracic legs dragging the case