ABSTRACT

Biological Studies of the Vesicular-Arbuscular (VA) Mycorrhizal Fungi *Gigaspora decipiens* and *G. rosea*

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The biological studies on VA mycorrhizas and VA mycorrhizal fungi presented in this thesis comprise experiments to evaluate the development of a transformation system for *Gigaspora*. A reliable and consistent method of *Gigaspora* spore germination was developed using commercial preparations of cellulase and a concentration of 3.2 units ml^{-1} gave optimum germination rates for *G. decipiens* and *G. rosea*. The possibility that cellulase may act as a germination signal in the soil for VA mycorrhizal spores is discussed.

Nuclear behaviour and nuclear division were observed in VA mycorrhizal fungi. VA mycorrhizal hyphae of *G. decipiens* and *G. rosea* representative of different stages of preinfection, intraradical and extraradical growth were examined. Observations of changes in nuclear shape suggest that mitosis is absent in preinfective hyphae and may occur in intraradical and extraradical hyphae. Attempts to promote mitosis in preinfection hyphae were unsuccessful. The role of the plant in VA mycorrhizal fungal mitosis is discussed.

To determine the effect of VA mycorrhizal colonisation on mitosis in the VA mycorrhiza, meristematic cells representative of the different stages of mitosis were scored from VA mycorrhizas of *Allium cepa*, for different levels of fungal colonisation. It was found that VA mycorrhizal colonisation reduced the root mitotic indices. Increasing levels of colonisation led to greater reduction in the mitotic indices of the mycorrhizas. The hypothesis that VA mycorrhizal fungi may "hijack" chemicals from the plant is discussed.

The sensitivity of spores of *G. decipiens* and *G. margarita* to antibiotics and benomyl was tested to determine an appropriate resistance gene that could be used in a transformation vector. The results showed that both species of *Gigaspora* were sensitive to hygromycin. A plasmid, SPC1 encoding hygromycin resistance under the control of the ascomycete promoter *trp* C was constructed.

An attempt to isolate a *Gigaspora* promoter, to replace the *trp* C promoter of SPC1 was made. A transformation vector with a zygomycetous promoter may be necessary for successful integration and expression in the fungal genomic DNA. Two strategies to identify and isolate the promoter of the *Gigaspora* actin gene and their limited success are discussed in detail.

The final chapter summarises the findings of the preceding studies and uses the information from these studies to evaluate the feasibility of developing a transformation system for VA mycorrhizal fungi. The implications of transgenic VA mycorrhizal inoculum use in the field are discussed.