Characterization of two genes, trehalose-6-phosphate synthase/phosphatase and nucleotide binding protein, shown to be differentially regulated in roots of *Cypripedium parviflorum* var. *pubescens* grown with a mycorrhizal fungus *Thanatephorus pennatus*

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Abstract

The analysis of gene changes associated with formation of the mycorrhizal symbiosis between orchid and fungi could have broad implications for plant pathogen interactions. Fungi associated with North American terrestrial orchids were once included in the pathogenic genus *Rhizoctonia*. This suggests that orchids are able to overcome or utilize normally pathogenic pathways to establish symbioses. A differential display technique was employed to analyze gene changes in orchid in response to a fungus. Samples of RNA from roots of *Cypripedium parviflorum* var. *pubescens* (CyPP) grown in the presence or absence of a mycorrhizal fungus; *Thanatephorus pennatus*, were analyzed using AFLP differential display. Forty-four fragments were selected out of 5000 as being differentially expressed, but only 15 sequences were obtained. Most showed homology to ribosomal genes. Two represented genes believed to be regulated by the mycorrhizal interaction: trehalose-6-phosphate synthase/phosphatase (*Tps*), which showed down-regulation and nucleotide binding protein (*NuBP*), which showed up-regulation. The *Tps* partial clone identifies 2100 bp at the 3' end of the gene and encodes a protein of 667 amino acids. The *NuBP* gene is approximately 1200bp in length and encodes a protein of 352 amino acids. The *Tps* gene exists in multiple copies with high expression in roots and low expression in rhizomes and leaves. The *NuBP* gene exists as a single copy and has a low level of expression in rhizomes and leaves. Expression of *Tps* is induced by sucrose, but reduced by trehalose. Cultivation of CyPP with non-mycorrhizal fungi did not affect expression of *Tps* or *NuBP*. Trehalose induced *NuBP* expression whereas sucrose did not. A second species of mycorrhizal fungi induced expression of *NuBP* but reduced expression of *Tps*. Analysis of *Tps* expression in *Arabidopsis* was done using promoter:*GUS* fusions. The *Tps* promoter:*GUS* plants revealed that *Tps* expression is constitutive in roots. Regulation of *Tps* driven *GUS* is expressed throughout seedlings. *GUS* was not detected in leaves of older plants but was detected in anthers and stigmatic surfaces of flowers. Expression of *GUS* driven by *Tps* showed a strong wound response and was present in the junction between siliques and pedicels.

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Dedication

I would like to dedicate this dissertation to my father, Dr. Ian Watkinson. He instilled in me the great love I have for the natural world and taught me to be curious about its many intricacies. His achievement 30 years ago has always been an inspiration to me. His many achievements since then continue to be inspirations. I hope that I can be such a mentor to my children so that they too can enjoy the wonders of this world on which we live.

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Two roads diverged in a wood, and I,
I took the one less traveled by,
And that has made all the difference.
- Robert Frost

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