

Figure 3-7. Analysis of tissue level expression of *NuBP* and *Tps*.

Seedlings of *Cypripedium parviflorum* were grown in perlite under normal conditions. Plants were removed from perlite, rinsed briefly and cut into rhizome (Rz), root (Rt) and leaves (Lf). RNA was extracted from each tissue and reverse-transcribed. PCR was performed on 0.125 ng of cDNA using primers specific for each gene (Table 3-1). PCR fragments were separated on 1% agarose gel and visualized with ethidium bromide staining and UV light. Actin was used to normalize template.

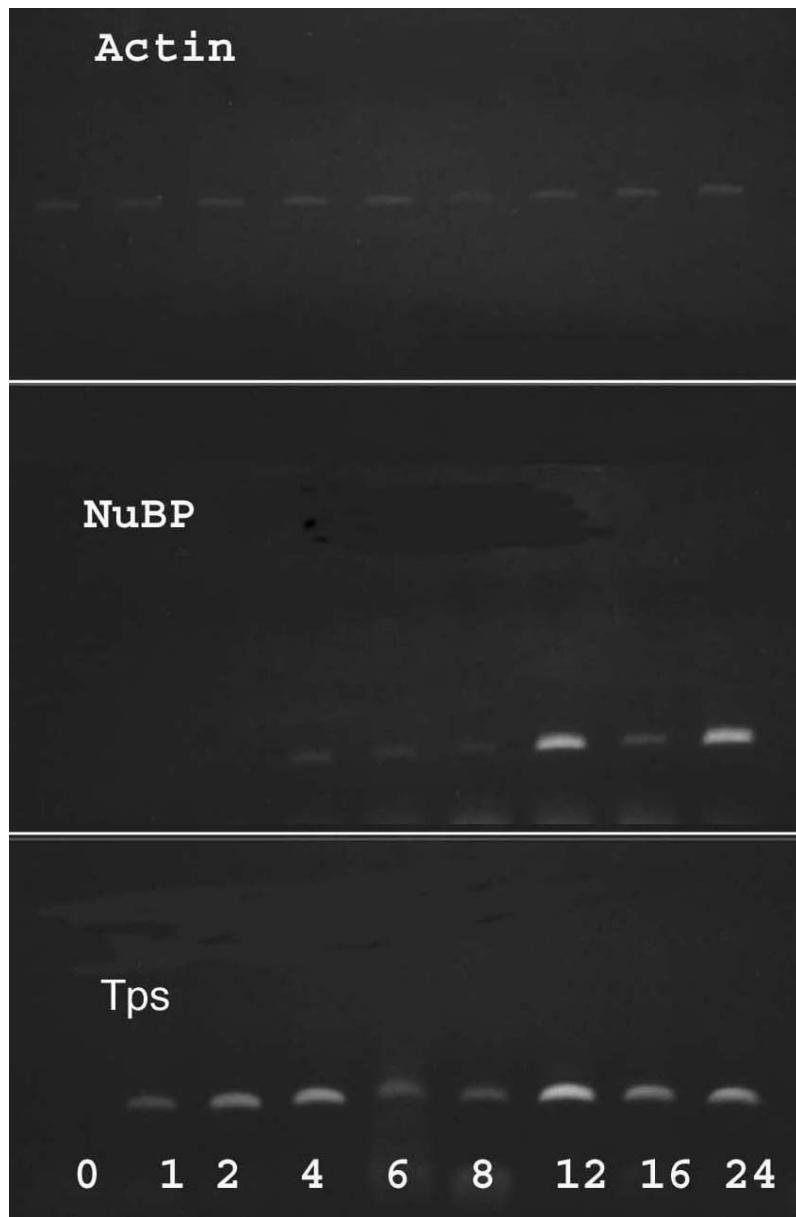


Figure 3-8. Expression of *Tps* and *NuBP* in roots of *Cypripedium parviflorum* in response to exposure to *Thanatephorus pennatus*.

Plants were grown in the absence (0) or presence of a fungus for 1-24 hours. RNA was extracted and reverse transcribed. PCR was performed on 0.125 ng of cDNA with primers specific for each gene (Table 3-1). PCR fragments were separated on 1% agarose gel and visualized with ethidium bromide staining and UV light. Actin was used to normalize template. Numbers below lanes indicate the amount of time orchid roots were cultivated with the fungus.

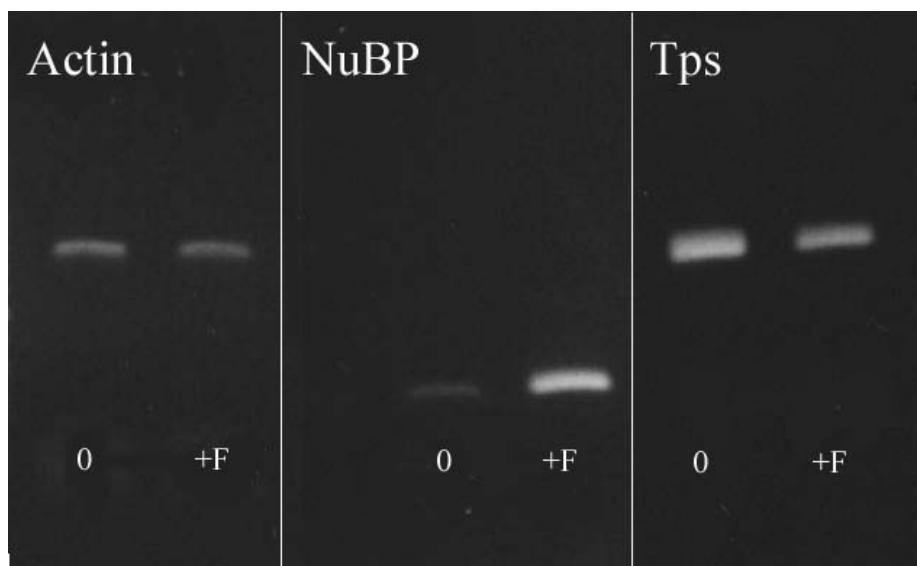


Figure 3-9. Expression of *NuBP* and *Tps* in response to *Ceratorhiza goodyera-repentis*. Plants of *Cypripedium parviflorum* were incubated on plates of oatmeal agar which were covered with mycelium of *Ceratorhiza goodyera-repentis*(+F) for 24 hours or were left untreated (0). Reverse transcription was performed after RNA extraction from root tissue. PCR was performed on 0.125 ng of template with primers specific for each gene (Table 3-1). PCR fragments were separated on 1% agarose gel and visualized with ethidium bromide staining and UV light. Actin was used to normalize template.

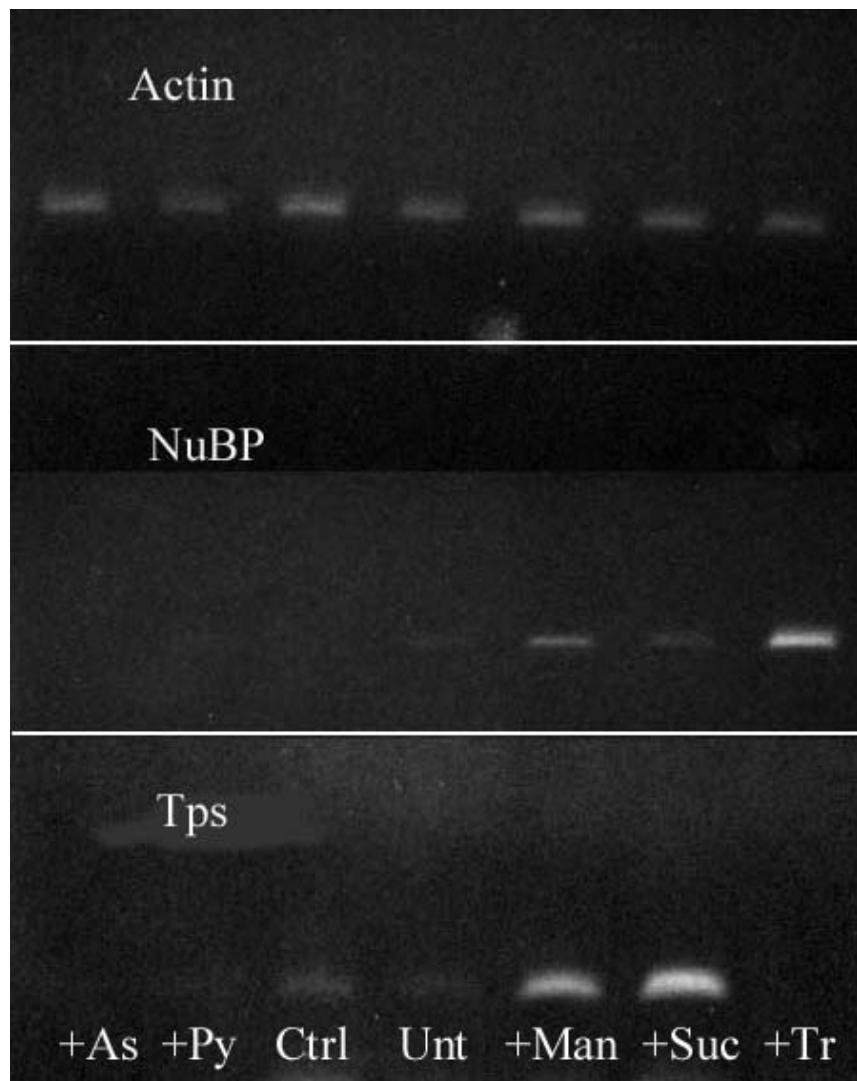


Figure 3-10. Expression of *Tps* and *NuBP* in response to various sugars and fungi. Seedlings of *Cypripedium parviflorum* were grown in the presence of various sugars: 50 μ M mannitol (+Man), 50 μ M sucrose (+Suc) and 50 μ M trehalose (+Tr). Seedlings were also grown with a non-mycorrhizal ascomycete (+As) and a general plant pathogen *Pythium ultimum* (+Py). RNA was extracted from roots of seedlings after 24 hours of exposure and was reverse transcribed. PCR was performed on 0.125 ng of template with primers specific for each gene (Table 3-1). PCR fragments were separated on 1% agarose gel and visualized with ethidium bromide staining and UV light. Template was normalized with Actin. Ctrl: control plants grown on oatmeal agar for 24 hours. Unt: untreated plants grown in perlite.

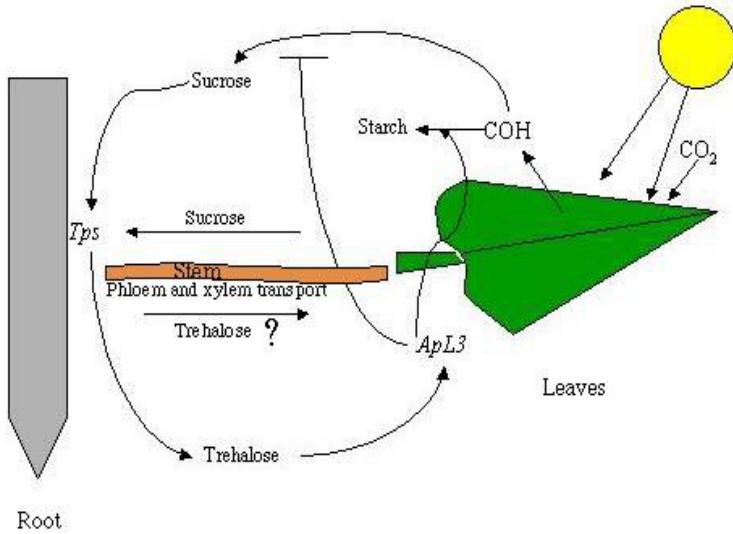


Figure 3-11. Model of trehalose as a sugar signal in carbon partitioning. Sucrose is produced in leaves and transported to roots via the phloem. Sucrose induces *Tps* leading to trehalose accumulation in roots. Trehalose is transported (mode of transport is not known though possibly a second messenger is involved) to stems where it induces the ADP-glucose pyrophosphorylase gene *ApL3* leading to starch accumulation. This reduces sucrose transport to the roots.