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# MOLECULAR MARKER GENES FOR ECTOMYCORRHIZAL SYMBIOSIS

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## ABSTRACT

Mycorrhizal symbiosis is a mutually beneficial association very commonly found among most vascular plants. Formation of mycorrhiza happens only between compatible partners and predicting this is often accomplished through a trial and error process. We investigated the possibility of using expression of symbiosis specific genes as markers to predict the formation of ectomycorrhiza. We used antibodies and cDNA probes corresponding to PF6.2 and Lbras genes of *Laccaria bicolor* to detect presence and expression of similar genes in other ectomycorrhiza fungi during their association with the red pine. Analyses using transmission electron microscopy and northern hybridization showed that such genes were expressed in several other fungi forming mycorrhiza with red pine. The results suggest that these genes may have a role in many ectomycorrhizal symbiosis systems and may serve as molecular markers for monitoring symbiosis development.

**KEYWORDS**: **Ectomycorrhizal fungi, symbiosis, *Laccaria bicolor*, molecular markers.**

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# INTRODUCTION

Most vascular plants, including a variety of conifers, establish mutually beneficial symbiotic relationships with ectomycorrhizal fungi that colonize their roots[1,2]. The interaction between the compatible plant and fungus pair leads to the formation of a new complex symbiotic organ called the ectomycorrhiza. The fungi penetrate the root epidermis and grow intercellularly between cortical cells forming a network of hyphae. The structure includes the innermost Hartig-net in the root cortex, the fungal sheath or mantle on the surface of the root, and the hyphae extending into the soil[3]. Extensive nutrient exchange takes place between the plant and fungal partners through this ectomycorrhizal organ[2,3]. The fungus acquires carbon from the plant in the form of hexoses[4,5,6] while the plant obtains minerals through the fungus by mobilizing substrates[7,8,9]. By extending the absorptive capabilities of the root system, the mycorrhizal association has been shown to improve plant's ability to tolerate biological and environmental stresses, including challenge by pathogenic fungi, heavy metal pollution, excessive erosion, drought, and reduced pH of the soil. The ectomycorrhizal phenomenon, essential for health and survival of many conifers and hardwood species, has been studied widely from ecological and physiological perspectives. A myriad of biochemical events happen leading to the initiation, formation and establishment of a functioning mycorrhiza. These events include turning on and off of several genes in the fungus as well as in the host plant[10,11]. Presumably, the root cells produce certain elicitors that induce expression of fungal genes involved in ectomycorrhiza establishment[10,12,13]. These include genes responsible for triggering attachment and developmental processes leading to the formation of Hartig net and hyphal mantle[14,15]. In addition, certain fungal genes involved in eliciting host plant defense reaction may be turned off to initiate the association process. Although all the molecular mechanisms involved have not been understood, studies undertaken in last couple of decades have identified several factors from the fungus that are involved in the initiation and establishment of ectomycorrhizae[16,17,18,19].

Our previous work has identified several genes from *Laccaria bicolor* that are turned on in response to interaction with red pine roots[16,17,20,21,22]. Using an *in vitro* interaction system we observed drastic changes in the gene expression of the fungal partner in which a number of genes were activated. Among several genes characterized using this method, the PF6.2 and Lbras genes have been appeared to be very important since the degree of change in expression level was significant. Moreover, expression of these genes was required for initiation, establishment as well as maintenance of the functioning mycorrhiza[16,17]. The Lbras was closely related to *ras* family of genes, which have been shown in other systems to be involved in signaling pathways controlling cell growth and proliferation. The function of the PF6.2 gene is unknown;its; sequences did not match to anything reported and deposited in the Genbank, although it showed some similarity to signal transduction genes. However, PF6.2 was one of the most abundantly expressed genes in response to exposure to plant roots. Since mycorrhizal symbiosis is a unique process involving formation of a new organ common to plant and fungus, it is possible that PF6.2 represents a unique gene not found in other systems reported. The complete genome sequence of the *L. bicolor* reported recently confirms that symbiotic fungi such as *L. bicolor* contain several unique genes not found in other organisms[18]. Temporal expression studies showed that both PF6.2 and Lbras were induced very early during interaction with the plant roots and continued to be expressed in established mycorrhizas. Transmission electron microscope (TEM) analyses using Lbras and PF6.2 protein antibodies showed that these proteins were present in both mantle and Hartig-net tissues and were localized near the cell wall[16,17]. Although many plants associate with ectomycorrhizal fungi, it is not possible to predict

which plant species will form ectomycorrhiza with a given ectomycorrhizal fungus. There appears to be a "compatibility" factor for successful formation of the symbiotic unit. However, there are no known quick methods to determine this compatibility and, often, this is accomplished by a trial and error method. In order to investigate whether genes like PF6.2 and Lbras are specific only to *L. bicolor* X red pine system or present in all mycorrhizal symbiosis systems, we undertook the present study. Our preliminary studies using antibodies to *L. bicolor* PF6.2 and Lbras proteins had suggested that similar proteins may be present in other mycorrhizal fungi associating with red pine. Therefore, we looked at expression of these genes in five other mycorrhizal associations with red pine. Our results indicated that such genes were indeed activated in other systems also, suggesting that PF6.2 and Lbras have a role in all ectomycorrhizal symbiosis systems and may serve as molecular markers for monitoring symbiosis development.

## MATERIALS & METHODS

### MEDIA AND CULTURES

The fungal cultures of *Amanita rubescens, Laccaria laccata, Suillusluteus, Suillusamericanus, Rhizipogon rubescens*and*Laccaria bicolor* were isolates associated with the roots of red pine obtained from Michigan's Upper Peninsula (provided by D. Richter, School of Forestry, Michigan Technological University, Houghton). The fungi were grown and maintained on MMN medium as previously described[20,23].

### *Formation of mycorrhizae*

This was done essentially as described earlier[21,24]. Briefly, red pine (*Pinus resinosa*) seedlings were grown in a sterile container (450 ml) by planting seeds in a 3:1 vermiculite: sphagnum peat containing 0.1X M4N medium without glucose. After the seedling had grown for ~ 3months and developed transverse roots, fungal culture was introduced by adding a 30 ml aliquot of liquid inoculum. The latter was prepared by placing mycelial pieces grown on M4N agar plates for 2-3 weeks at $24^0$ C in a 1 L M4N media contained in a 2 L Erlenmeyer flask and incubating at room temp in the dark for 2-3 weeks. The media was then macerated using a Tissumizer homogenizer for 30 sec and used as an inoculum. About 3 months after inoculation, roots were examined under microscope for the presence of mycorrhizae. The mycorrhizal tissues were collected and used for TEM analyses as well as for isolation of RNA for northern analyses. A portion of the tissue was used for isolating DNA and to identify the fungus present in the ectomycorrhizae.

### *DNA isolation, PCR and Identification of the fungus*

The ectomycorrhizal tissues were used to isolate fungal DNA and identify the fungus through ITS-region sequence as described earlier[25,26]. Briefly, about 10 mg tissues were homogenized in extraction buffer using a bead beater for 3 min and DNA extracted using QIAgen'sDNAeasy Plant Mini Kit. The DNA was used in a PCR using primers that amplify the highly variable internal transcribed spacer (ITS) region of the fungal ribosomal DNA. The PCR product was either used for nucleotide sequencing directly or was cloned first and then sequenced. By comparing the sequence obtained with those present in the Genbank the identity of the fungus was ascertained[27].

### *Protein and Antibodies*

Antibody production to Lbras protein has been described earlier[17]. The antibodies to PF6.2 protein were produced using a similar method. Briefly, the cDNA fragment corresponding to PF6.2 was cloned into the expression vector pet22b+ (Novagen), which facilitated expression of a fusion protein containing a 3'-histidine tag[28,29,30]. After transformation of *E. coli* with this construct and expression of proteins according to the manufacturer's instructions, the PF6.2 fusion protein was purified from cell extracts through affinity chromatography using the His-Bind Metal Chelation resin (Novagen). Polyclonal antibodies to the fusion proteins were generated

in rabbits as described earlier[17,31]. The antibodies were tested for their specificity through western blot analysis[17].

***Immunolocalization with Transmission Electron Microscopy (TEM)***

Sample preparations for TEM analyses[32] were similar to what was described earlier[17]. Briefly, small (< 4 mm) mycorrhizal and nonmycorrhizal root pieces were cut and fixed in 2.5% glutaraldehyde present in buffer NaP (10 mM sodium phosphate, pH 7.2) by incubating for 16-20 h at 40°C. They were then placed under vacuum at room temperature for 2 h. After washing (3 × 15 min) with NaP buffer, samples were treated with 1% osmium tetraoxide for 1 h and washed with water. Water content of the samples was gradually removed by successive washes with 30% ethanol, increasing the ethanol concentration gradually. After a final treatment with 100% ethanol, infiltration withLR white resin was performed[32]. An ultramicrotome equipped with a diamond knife was used to get cross sections of roots (100 to 120 nm) on nickel grids that were subjected to immunological analysis. After an initial blocking step in TBS (50 mMTris-HCl, pH 7.6, containing 0.5 M sodium chloride) containing 1% bovine serum albumin (BSA) and 1:30 dilution of rabbit preimmune serum, the grids were incubated for 16 to 20 h with 1:1000 dilution of anti-Lbras antiserum or anti-PF6.2antiserum in TBS at room temperature. After washing with TBS, they were treated with TBS containing 1% BSA and 1:20 dilution of gold-labeled second antibody (goat antirabbit immunoglobulin Polygold 10 nM; Polysciences, Warrington, PA, U.S.A.). After incubation for 1 h, root sections were washed several times with TBS followed by water and dried. Some of the samples were subjected to post-staining with uranyl acetate[32]. The grids were then examined with a JEOL JEM-1010 TEM operated at 80 kV.

***Northern analyses***

Mycorrhizal root tissues were ground to a fine powder in liquid nitrogen and total RNA was isolated using the Trizolkit (Gibco BRL, MD) as per manufacturer's instructions[16]. Northern analysis was performed essentially as described earlier[16,17]. Total RNA (10 □g) was electrophoresedon 1% agarose gels and transferred to Hybond-N membranes(Amersham Pharmacia Biotech, Piscataway, NJ).Gels were stained withSYBR Green to determine equal loadings and intensity ofRNA.The cDNA fragments of Lbras and PF6.2 cDNA clones were isolated and purified by agarose gel electrophoresis followed by extraction of DNA using the QIAEX gel extractionkit (Qiagen Inc., CA). About 25 ng of purified cDNAwas labeled with $^{32}$P-dCTP (3000 Ci/mmol, Amersham Corp, NJ) using the DECAprime II DNA Labeling Kit (Ambion, Austin, TX,U.S.A.). These were used as probes in hybridization analyses ofthe membrane-bound nucleic acids[33,34,35]. Prehybridization, hybridizationand washings were done as described before[16].

## RESULTS

The PF6.2 gene expression in *L. bicolor*is initiated very early during interaction of the fungus with the red pine roots. The PF6.2 mRNA encoding a protein of about 47 kDwas ~ 1.5 kb in length and could be detected as early as 6 h into interaction[16]. The Lbras mRNA from *L. bicolor* on the other hand was detectable only 48 h after interaction with roots. The Lbras mRNA was also ~ 1.5 kb in length, but encoded a protein of about 26 kD[17]. However, both PF6.2 and Lbras continued to be expressed in established mycorrhizas in *L.bicolor* X red pine. Therefore, we used established mycorrhizal tissue for analyses in these experiments.

***1. Ectomycorrhiza formation***

The ectomycorrhiza synthesis performed with red pine seedlings under semi-sterile conditions is shown in Figure 1. Seedlings were typically about 7-10 cm in height and had developed lateral roots 3 months after germination. After inoculation and formation of ectomycorrhiza (about 3 months later), their height was about 12-15 cm. The ectomycorrhizal association was not widespread on the root

**Figure1**

***Synthesis of ectomycorrhiza: Red pine (Pinus resinosa) seeds were germinated in vermiculite medium in a sealed container under semi-sterile conditions. After ~3 months, when the seedling developed lateral roots, the ectomycorrhizal inoculum was introduced and incubated for ~ 3 months for ectomycorrhiza to be formed.***

system under the conditions we used (only about 15-25%). We checked the roots under the microscope to select portions of tissue for further analysis. In order to confirm that the fungus in the ectomycorrhizal tissue was the one that was used for inoculation, we reisolated the fungus from the tissue and were subjected to morphological analysis.

***2. Antibodies and transmission electron microscopy***

Based on the nature, expression pattern, and the level of expression of PF6.2 and Lbras in *L. bicolor*, we had predicted that similar genes may be present in other ectomycorrhizal systems. Our preliminary studies using the antibodies to *L. bicolor* PF6.2 showedthat such proteins were indeed present in other ectomycorrhizal fungi. We confirmed this by performing immuno-dot blot analysis using ectomycorrhizal tissues of red pine with the fungi *Amanita rubescens,*

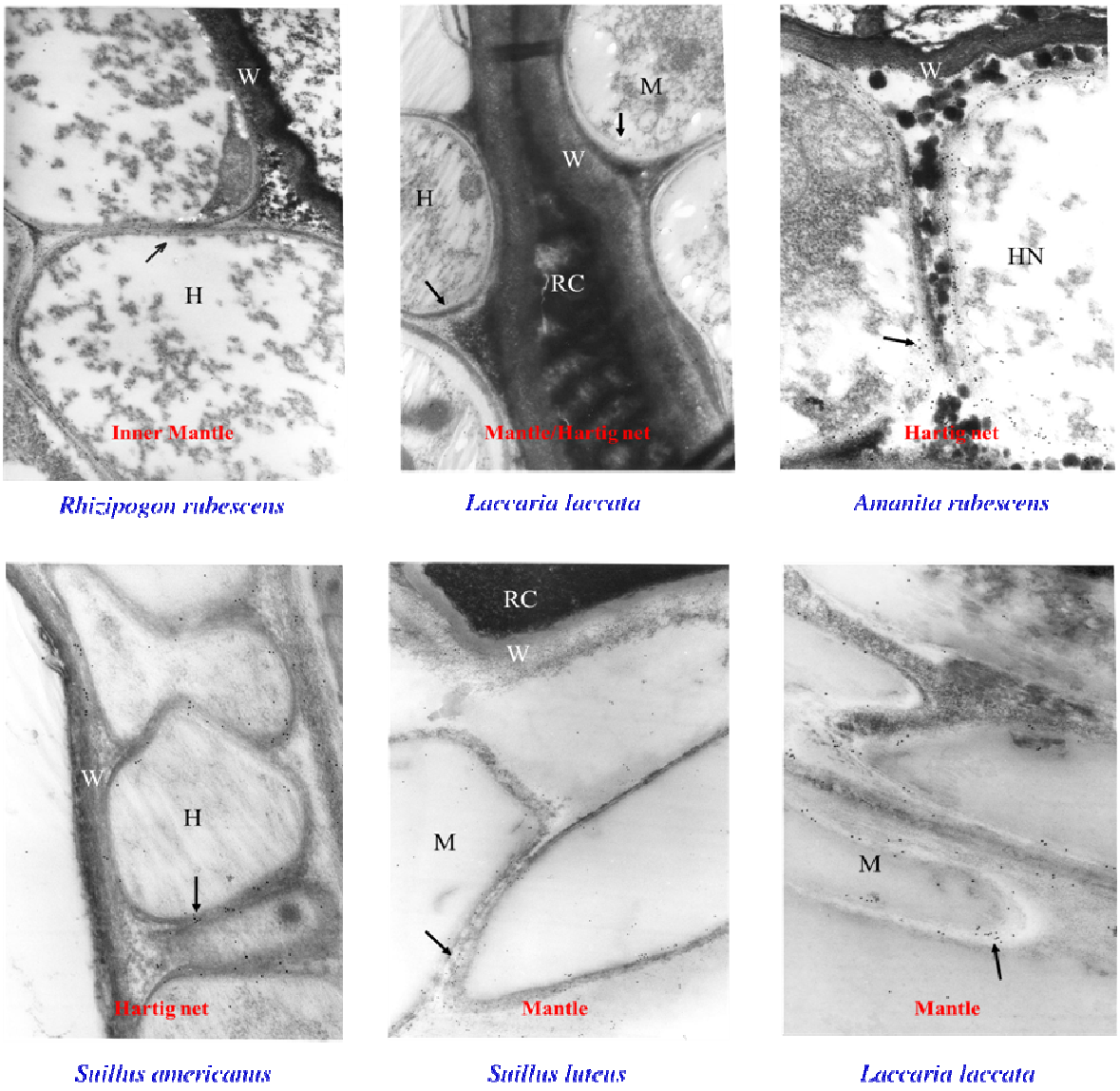


**Figure 2**

***Analyses with antibody to the Lb PF6.2 protein. TEM photographs of mantle and Hartig-net hyphae from fungal X red pine ectomycorrhizas. The fungus and the tissue type are indicated on each picture. Immunoreactive material indicated by gold label (seen as black dots) was localized primarily near fungal cell walls (arrows). H- Hartig-net hypha; M- Mantle hypha; W- Root cell wall; RC- electron-dense root material.***

*Laccaria laccata, Suillusluteus, Suillusamericanus and Rhizipogon rubescens* (data not shown). All these fungal extracts showed presence of proteins that cross reacted with antibodies to Lbras and PF6.2. Therefore, we used these antibodies in TEM analyses to localize their presence in ectomycorrhizal tissues. The TEM results obtained from immunolocalization of PF6.2 in mycorrhizal tissues are shown in Figure 2. The gold label, which indicates the presence of the immunoreactive protein, is seen as an opaque dot in the TEM analysis (shown by arrows). The analyses indicated that in all the tissues examined there was a protein cross-reacting with the PF6.2 antibodies. Although the figure shows data obtained with only some of the tissues, we observed proteins cross reacting

with the antibodies in all ectomycorrhizal tissues from *Amanita rubescens, Laccaria laccata, Suillusluteus, Suillusamericanus and Rhizipogon rubescens*. This included: 1) the Hartig-net (H), the network of hyphae penetrating in the interstitial space of the cortical cells of the roots, and 2) the mantle (M) where the fungal hyphae are on the surface of the roots and extending from there. In all cases, the immunoreactive material was primarily localized near the cell wall as observed earlier in the case of *L. bicolor*[17]. However, in case of nonmycorrhizal tissues (only root) treated and observed in a similar fashion, no immunoreactive material was detected (data not shown). A similar pattern was seen in TEM analyses using the Lbras antiserum (Figure 3). The figure shows only some of the data obtained with antibodies to Lbras protein. However, both mantle and Hartig-net tissues of all the fungi tested had the immunoreactive material. Also, as in the case of PF6.2, the label was mostly present near the cell wall (indicated by arrows).

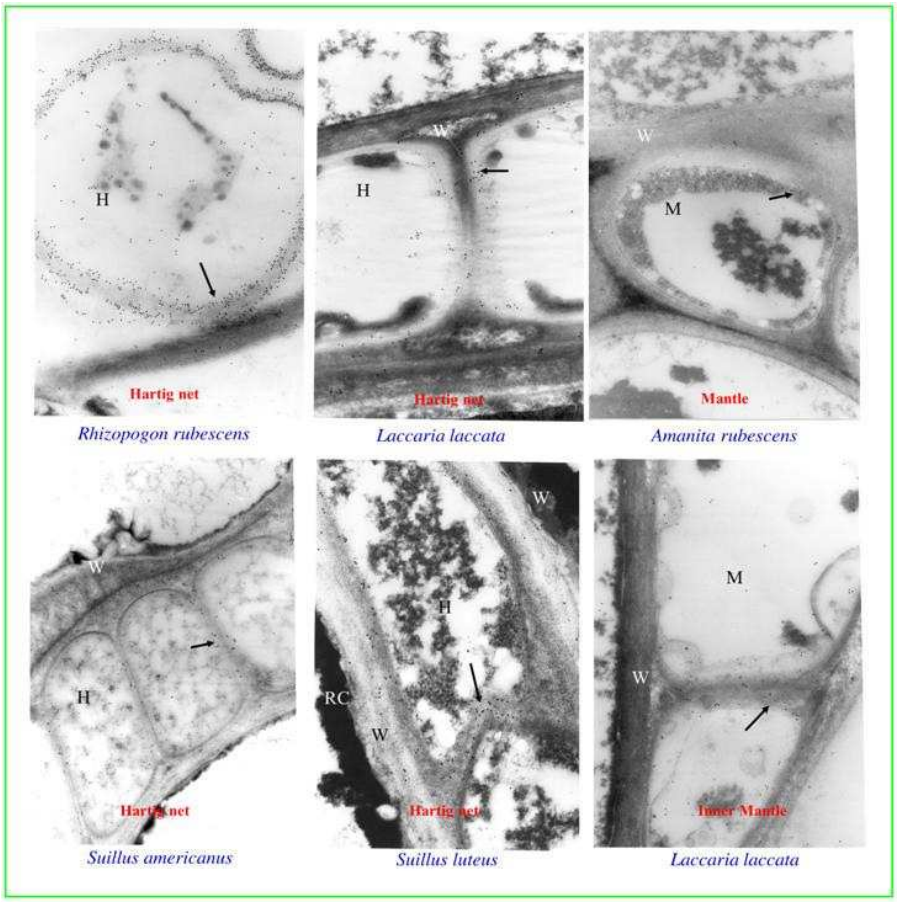


**Figure 3**

***Analyses with Antibody to the Lbras protein. TEM photographs of mantle and Hartig net hyphae from fungal X red pine ectomycorrhizas. The fungus and the tissue type are indicated on each picture. Immunoreactive material indicated by gold label (seen as black dots) was localized primarily near fungal cell walls (arrows). H- Hartig net hypha; M- Mantle hypha; W- Root cell wall; RC- electron-dense root material.***

We also looked at the presence of respective mRNAs in the ectomycorrhizal tissues. Similar to antibodies, our preliminary studies using dot blot hybridizations had shown that *L. bicolor* cDNAs probes were able to hybridize to RNA in other fungi, presumablytheir counterparts (data

not shown). Our earlier work had also shown thatwhile PF6.2 mRNA was detectable in *L. bicolor*as early as 6 h after interaction, the Lbras mRNA was observed only 48 h after interaction. However, both continued their expression in the established mycorrhiza. Therefore, we decided to use the completely formed mycorrhizal tissues for these analyses. Also, we used total RNA from each tissue for analysis since separating poly(A) RNAwould have required larger quantities of tissue, which was not available in this study. Also, the sensitivity of the technique was adequate to detect the corresponding sequences in the total RNA[16,17]. However, considering low degree of homology that may exist among cDNA/mRNA sequences, we sued less stringent conditions for hybridization analyses. Figure 4 shows the northern analysis results obtained using the PF6.2 cDNA fragment as the probe. The lower panel shows the SYBR Green II stained gel picture before nucleic acids were transferred to the Hybond membrane and the top panel shows the autoradiogram of the hybridized membrane. As expected, we were able to detect the ~ 1.5 kb PF6.2 mRNA from *L. bicolor* as reported earlier[16]. The probe also hybridized to a 1.5 Kb RNA present in each of the fungal RNA analyzed. The size of the band in each case was similar to the size of the PF6.2 mRNA from *L. bicolor*. While the relative intensities of hybridization signal were same among most RNA samples, only in case of *R. rubescens*it was lower. This was not due to the total amount of RNA used in the analysis as the stained gel indicated that all the lanes had similar amounts of RNA. The observed result was probably because of *R. rubescens* PF6.2 having a lower degree of homology to *the L. bicolor* PF6.2.

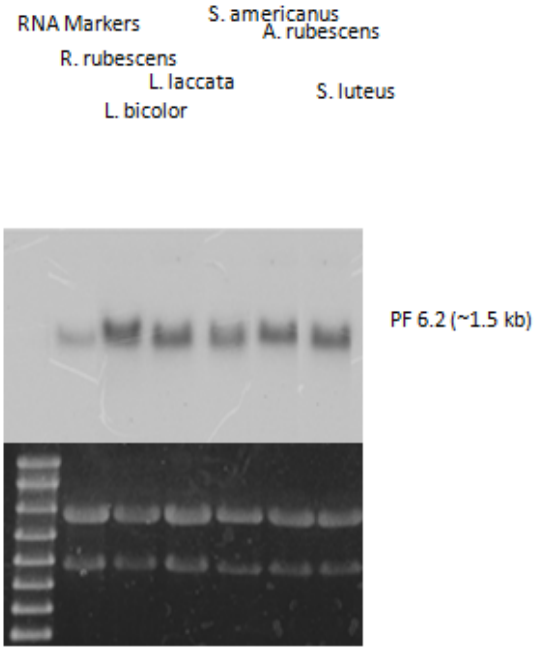


**Figure 4**

***Northern analysis using PF6.2 cDNA probe.TotalRNAwas(10□g) waselectrophoresed on 1% agarose gels and RNA bands were visualized by staining with SYBR (Lower panel). The bands were transferred to a Hybond membrane and subjected to hybridization using labeled PF6.2 cDNA probe as described in Materials and Methods. RNA size markers used in the analysis can be seen in the left lane.***

We also looked at the *ras* mRNA levels in these tissues using the Lbras cDNA fragment as probe in northern analysis (Fig. 5). Results were very similar to those observed for the PF6.2 northern analysis. In case of *L. bicolor*, the probe hybridized to the 1.5 kb Lbras mRNA as reported earlier (Sunderam et al 2001). The probe also hybridized to a ~1.5 kb RNA present in each mycorrhizal tissue, suggesting that these tissues had an equivalent of Lbras mRNA which was being expressed in the functional ectomycorrhiza. Moreover, as seen with PF6.2 mRNA, the Lbras mRNA also appeared to have less homology to its counterpart in *R. rubescens*. The signal was much lower than others, although same amounts of total RNA were used for the analyses.

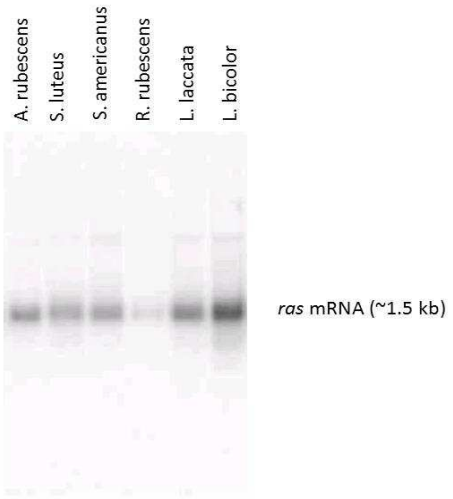


**Figure 5**
***Northern analysis using Lbras cDNA as probe. Total RNAs from each of the mycorrhizal tissue was subjected to northern analysis as described in Materials and Methods. For details see legend to Figure 4.***

## DISCUSSION

The mycorrhizal symbiosis represents an unique system where two different organisms interact to form an entirely new organ mycorrhiza, which then becomes common to both organisms. Both root cells and fungal cells are integrated in this organ and function together to transport nutrients to each other. It is conceivable that such a complex symbiotic unit would involve genes and mechanisms that are very distinctive. Recently, entire genome sequences of two symbiotic fungi *Laccaria bicolor* and *Tuber melanosporum* were reported; this study was undertaken because of the importance of mycorrhizal symbiosis in agriculture and forestry. Initial analysis of the genome revealed several unexpected features in the *L. bicolor* genome which contained a battery of unique effector-type small secreted proteins with unknown function[18]. Further analysis showed that at least one such protein MISSP7, expressed in high copy numbers during interaction with the plant roots, may be essential for symbiosis development[36]. It appears similar genes are required for initiation, development and maintenance of the mutualistic symbiosis[37]. Our interest was to see whether we could identify and usesimilar symbiosis specific genes that may be common

among all mycorrhizal systems and their possible use as markers for monitoring symbiosis development. We chose PF6.2 and Lbras genes for this study based on our observations of their expression during initiation and establishment of mycorrhiza symbiosis in case of *L. bicolor* X red pine[16,17,21]. Similar to the MISSP7, the PF6.2 and Lbras were expressed at high levels during interaction with plants. PF6.2 was one of the most abundantly expressed genes among all the symbiosis-specific genes we looked at during interaction of *L. bicolor* with red pine roots. It was expressed as early as 6 hours after exposure to roots in the *in vitro* interaction studies[20,21]. Moreover, the expression continued even during established/functioning mycorrhiza indicating that it serves an important function in mycorrhizal symbiosis. It was a unique gene not related to any other genes found in other systems.The complete nucleotide sequence of the PF6.2 gene revealed an unusual structure, which had multiple repeats within their exons as well as introns. Presence of such repeats suggested that the PF6.2 may have a function similar to some of the genes associated with signal transduction and recognition domains of receptors as well as metal binding and transport proteins[38,39,40]. Based on all observations it was clear that not only its expression was symbiosis specific, it probably played an important and critical role in the establishment and maintenance of the symbiosis.Similarly, the Lbras belongs to *ras* family of genes, which are known to be associated with signaling pathways controlling cell growth and proliferation. Recent work on *L bicolor* genome has shown that it belongs to a group of several RAS GTPases, which are key components of signal transduction pathways[37]. It was not surprising that sucha gene is involved in the development of mycorrhiza. The Lbras from *L. bicolor* was closely related to mammalian *ras* genes and was able to complement the *ras* 2 in *S. cerevisiae*[17]. Considering the characteristics of PF6.2 and Lbras, we thought similar genes would most likely be present in all mycorrhizal fungi and are required for mycorrhizal symbiosis.

We used a sterile containerized system for generating the mycorrhizal tissues. We have consistently used this system to produce mycorrhizal tissues without problems of any contamination. In the present study, the ectomycorrhizal tissues we used for analysis indeed contained the same fungus that was used for inoculation. We conformed this by isolatingthe fungus back from the mycorrhizal tissues and performing morphological analyses. In other instances, we have used the same system where identification of the fungus was done by isolating the fungal DNA followed by analysis of the variable ITS region of the fungal ribosomal region[25,26,27]. Routinely, we found the same fungal species that was used in inoculation in such experiments. Our data clearly indicates that indeed the fungi tested here had similar proteins present which were closely related to their counterparts in *L. bicolor*. Since we were not able to isolate enough protein for generating antibodies, we had to resort to *in vitro* expression followed by purification of respective proteins in bacteria. The antibodies generated were specific to the protein, which was confirmed by western analysis. Antibodies did not cross react with any protein in root tissues alone and in *L. bicolor*that had not been exposed to roots, indicating that they were specific to respective proteins.Therefore, we employed these in TEM analyses. The gold spots indicative of the presence of respective proteins were present in both Hartig-net and mantle tissues in all the mycorrhizal tissues. Furthermore, as in the case of *L. bicolor*, these proteins were localized near the cell wall in the mycorrhiza[17]. In mammalian cells, *ras* proteins are localized near the plasma membrane and interact with tyrosine kinase receptors[41] to influence the cellular signaling pathways controlling critical cellular events. Since PF6.2 also appears to be related to signal transduction pathway genes, it makes sense that it too has to be localized near the cell wall and closer to the inter-cellular region in the ectomycorrhizal tissue. Taken as a whole, it

appears that as in *L. bicolor*, the counterparts of the PF6.2 and Lbras in other fungi have similar function. Presence of homologous genes in other fungi was further confirmed by the northern analyses. It is not clear what degree of homology exists among the *L. bicolor* genes tested here and their counterparts detected from other fungi. In the TEM analyses, we could not see any quantitative differences. However, the northern analysis suggested that the homology was adequate to detect respective mRNAs using the technique. Though we used moderately stringent conditions for hybridization analyses, results showing a single specific band in each fungal RNA samples suggest that there is significant homology among *L. bicolor* cDNA and its counterparts in other fungi tested. However, this was partially true in the case of *Rhizopogon rubescens*. Both PF6.2 as well as Lbras cDNA probes hybridized weakly to their respective counterparts from this fungus (Figure 4 and Figure 5). However, overall similarities such as presence and localization of homologous proteins cross reacting with antibodies in TEM analyses as well as sequence similarities seen by northern analyses suggest that all the fungi tested have genes similar to PF6.2 and Lbras in their genome. Isolation of respective genes from these fungi and sequence characterization will be necessary to confirm this. There is a lack of basic biochemical knowledge describing what factors contribute to successful formation of symbiosis and, therefore, the success of reforestation through mycorrhizal phenomenon. Very often, the process of utilization of mycorrhizal fungi is a trial and error process. Adequate knowledge of relationships between soil conditions and tree/fungus interactions has not been worked out, which is very important for optimum utilization of the mycorrhizal phenomenon. Biochemical markers linking soil chemistry and individual/group of fungi that will be compatible under certain conditions will help in determining suitable fungi that will be successful in a specific region. At present, there are no biochemical methods to determine this compatibility. The studies described here suggest that PF6.2 and Lbras like genes have a role in all ectomycorrhizal symbiosis systems and are important for functioning of ectomycorrhiza. Such genes can be used for monitoring formation, development and functioning of ectomycorrhizas and will be useful in identifying and better predicting the tree/fungus combinations that would work under specific conditions.

## CONCLUSION

The fungi *Amanita rubescens*, *Laccaria laccata*, *Suillusluteus*, *Rhizopogon rubescens* and *Suillusluteus* appear to have similar proteins (PF6.2 protein and Lbras protein), which cross-reacted with the antibodies raised against their counterparts from *Laccaria bicolor*. The label was localized mainly near the fungal cell wall as observed in *L. bicolor* X red pine ectomycorrhizas. This was true for both the PF6.2 and the Lbras proteins antibodies.Northern hybridization analyses using cDNA probes corresponding to PF6.2 and Lbras showed that other fungi had similar mRNAs that were expressed in ectomycorrhizae with red pine roots.The results suggest that these genes may have a role in many ectomycorrhizal symbiosis systems and may serve as molecular markers for monitoring symbiosis development.

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