**PtSRR1, A PUTATIVE PISOLITHUS TINCTORIUS SYMBIOSIS RELATED RECEPTOR GENE IS EXPRESSED DURING THE FIRST HOURS OF MYCORRHIZAL INTERACTION WITH CASTANEA SATIVA ROOTS**

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**ABSTRACT**

*PtSRR1* EST was previously identified in the first hours of *Pisolithus tinctorius* and *Castanea sativa* interaction. QRT-PCR confirmed *PtSRR1* early expression and *in silico* preliminary translated peptide analysis indicated a strong probability that *PtSRR1* be a transmembrane protein. These data stimulate the *PtSRR1* gene research during ectomycorrhiza formation.

**Key words:** ectomycorrhiza, symbiosis related genes/proteins, *Pisolithus tinctorius*.

The formation of ectomycorrhiza is a process governed by a complex biochemical and molecular interaction between the two partners before physical contact. Several stages of the ectomycorrhiza formation and maintenance processes from preinfection to the formation of the mantle and the Hartig net have been described, and it is obvious that changes in gene expression have to accompany the processes leading to symbiosis (6,8).

Studies evaluating the fungal transcript pattern during symbiosis formation have demonstrated that mycorrhization induces changes in the expression of genes normally expressed in the free organisms, without the participation of symbiosis specific genes (9). In this paper, we present a fungal cDNA EST representing a gene that is upregulated at 12 h of interaction between *P. tinctorius* and *C. sativa* (1). Its expression, the putative protein structure and its possible function in the symbiosis are discussed.

Biological material acquisition/maintenance and ectomycorrhizal induction is described by Baptista et al. (2007) (2). Micorrhizal stimulated (“myc”) and control mycelium (only in water) were harvested 12 h after contact, snap-frozen in liquid nitrogen and stored at -80ºC. A cDNA library of *P. tinctorius* was constructed from 6 µg of mRNA mix (control and “myc”) using the SMART cDNA Library Construction Kit (BD Clontech, Palo Alto, CA, U.S.A) as presented by Acioli-Santos et al. (2008). For the quantification of the *PtSRR1* mRNAs, the reverse transcription of each target RNA (control RNA and “myc” at 6 h and 12 h of interaction) was carried out (7).

The cloned *PtSRR1* EST fragment is 432 bp long. An untranslated region is observed downstream from the putative open reading frame (Fig. 1). The *PtSRR1* sequence has 70% similarity to a sequence of *Pisolithus microcarpus* (CB010071), a fungus that forms ectomycorrhiza with *Eucalyptus*. The putative *PtSRR1* peptide has 48% similarity to a protein of the fungus *Schizophyllum commune* (AF335537) that is upregulated under low nitrogen conditions. The study of the *PtSRR1* expression using QRT-PCR allowed the confirmation of the up-regulation at 12 h of interaction, revealing positive transcription rates 1350 fold higher than the control. At 6 h of fungus-plant interaction, the relative values were close to one, suggesting that changes in the transcription levels may occur between 6 and 12 h of interaction.

*In silico* translation of *PtSRR1* nucleotide sequence resulted in a peptide fragment of 75 amino acids (8.2 kDa), without the

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PtSRR1, a putative P. tinctorius symbiosis

1  AGT  CGT  CTG  GGA  CAC  GAG  TAC  GCC  CCT  GCA  CAA  ATC  TCA  AAT  TCA  45
1  S  R  L  G  H  E  Y  A  P  A  Q  I  S  N  S  15
46  GAG  GGA  CAG  ATT  TAT  CTC  GTC  GTA  AAC  AAC  CTC  ATC  GAT  TGC  GAC  90
16  E  G  Q  I  Y  L  V  V  N  N  L  I  D  F  D  30
91  TAC  TTG  TTG  GCA  ATG  TCC  ATT  CTC  GAT  GCC  ATC  GTC  TTG  135
31  Y  L  L  A  N  D  F  N  I  L  D  G  S  V  M  45
136  GTC  ACA  GTA  CCG  GAC  GTG  CCG  ACT  GAC  ATT  TAT  GCC  ATC  GTC  TTG  180
46  V  T  V  P  D  V  P  T  G  I  Y  A  I  V  L  60
181  TTT  GGT  GAT  TCT  GGT  AAC  TTT  AGC  CAG  AAC  TCC  AC  ATC  ATA  GCG  225
61  F  G  D  S  G  N  F  S  Q  N  F  T  I  I  A  75
226  TGA  TCC  CAT  CAC  GTC  CTT  GCA  ACT  TTA  TCT  CTC  TGA  ACG  ATT  TCA  270
76  *  
271  TGA  ACA  ATG  ATG  AAG  GAC  TTC  TGT  TTC  GAT  CAC  TCA  GGA  CTT  315
316  GGT  TTC  ATA  CAT  TAG  GAC  GAC  AAA  TAC  ACA  TGA  CTC  GGA  ACA  TTT  360
361  AGC  AAT  GGA  CTT  GTA  ACC  CCC  TTT  CGC  ATT  CTG  CTG  TAC  GTA  TAT  405
406  GGA  CTA  GGA  TCC  GGG  ACC  ATT  CTA  CTA  432

**Figure 1.** The PtSRR1 EST: nucleotide sequence (432 bp) and partial ORF (letters below the codons, totalling 75 amino acids). The termination codon is assigned with an asterisk. The partial ORF was identified using MapDraw (Informatik Inc. USA) and represents the largest translation region for the sequence.

Initial methionine. No cysteine residues were found in the PtSRR1 amino acid sequence. The analyses of the PtSRR1 peptide primary structure (http://ca.expasy.org/cgi-bin/prosite) enabled the identification of four post-translational modification sites as follows: two N-glycosylation sites with high probability of occurrence between the residues 66 to 69 (NFSQ) and 70 to 73 (NFTI), and two Casein Kinase II phosphorylation sites, in the positions 13 to 16 (SNSE) and 47 to 50 (TVPD), respectively (Fig. 2a). No usual protein domains were identified. Secondary PtSRR1 structure analysis showed abundance of beta-structures (Fig. 2a). No helix was detected. The peptide shows a well-defined transmembrane region, despite the low probability suggested by its analysis (http://www.predictprotein.org). It was not possible to obtain a PtSRR1 three-dimensional model based on homology modeling (http://www.swissmodel.expasy.org/SWISS-MODEL.html) (Fig. 2b).

The expression of several genes at 6 h of interaction between *Laccaria bicolor* and *Pinus resinosa* has been reported (4,5). However, most of the differentially expressed fungal genes were observed in later stages of symbiotic development, especially after two or more days of interaction (3,6,9), which is corroborated by the 12 h *PtSRR1* transcription. Therefore, the high relative expression of *PtSRR1* at 12 h favours its investigation. QRT-PCR data confirmed the cDNA microarrays analysis of the fungal *PtSRR1* and its high relative transcription at 12 h of ectomycorrhizal interaction. Transcription of this gene does not occur until 6 h of contact, suggesting that this period between 6 and 12 h can be critical for its expression.

The *PtSRR1* gene is that probably triggered by the low availability of nitrogen that could function as an “indicator” of host root proximity. *PtSRR1* homologue peptide (AF335537) was identified in *Schizophyllum commune*. This homologue peptide presents high expression when the mycelium is growing under low nitrogen availability conditions. Further physiological studies and the acquisition of the complete ORF of this gene are necessary for functionality tests in the symbiosis. These results would allow to understand the real function of the *PtSRR1* protein.

As the *PtSRR1* amino acid sequence is not complete and the three-dimensional protein structure is not known, any conclusion about the role of this protein is premature. However, considering its secondary structure prediction, the *PtSRR1*
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seems to be a transmembrane protein with an intracellular segment containing at least one phosphorylation accessible site and an extracellular region containing two glycosylation sites. Thus, there is a possibility that the PtSRR1 acts as membrane receptor/extra-intracellular signal-transducer element through sites of glycosylation and phosphorylation, or be a secreted protein. However, in silico data obtained using the truncated PtSRR1 amino acid sequence would differ from the full-length amino acid sequence. These data strongly stimulate the research of PtSRR1 gene role in the ectomycorrhizal process as a potential marker/regulator of the early stages of symbiotic interaction.

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RESUMO

*PtSRR1*, um possível receptor simbiose-regulado de *Pisolithus tinctorius* é expresso nas primeiras horas de interação ectomicorrízica com raízes de *Castanea sativa*

*PtSRR1* foi isolado preliminarmente de *P. tinctorius* nas primeiras horas da interação com raízes de *C. sativa*. Análises de QRT-PCR confirmaram sua expressão positiva (12 h) e seu peptídeo putativo indicou forte possibilidade para proteína transmembranar. Estes dados estimulam o estudo do *PtSRR1* durante a formação de ectomicorrizas.

**Palavras-chave:** ectomicorriza, genes/proteínas simbiose-regulados, *Pisolithus tinctorius*.

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