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CLONING AND BACTERIAL EXPRESSION OF A PATHOGEN-INDUCIBLE ISOFORM OF β-1, 3-GLUCANASE GENE FROM *HEVEA BRASILIENSIS* WITH ANTIFUNGAL PROPERTIES

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A pathogen-inducible isoform of β-1, 3-glucanase gene (β-glu) from the Para rubber tree (*Hevea brasiliensis* Muell. Arg.) is reported for the first time. In order to induce the production of β-glu, two-week-old leaves of *H. brasiliensis* (clone RRII 105) were inoculated with abnormal leaf fall-causing pathogen, *Phytophthora meadii*. RNA was isolated from the near necrotic zones of the infected samples by LiCl precipitation. First strand cDNA was synthesized and sequence coding for the final mature β-glu (EC 3.2.1.39) was amplified. A functional cDNA clone was constructed using the pET 32a+ expression system. The major difference of the new basic isoform with a predicted pI of 9.26 is the absence of a glycosylation site at Asn-27, which is present in all the reported cDNA sequences of *Hevea* β-glu. Conditions were optimized for IPTG- induced over-expression of the gene in the *E. coli* strain BL21 (DE3), in soluble form. The column purified recombinant protein retained its functionality as proved by its ability to hydrolyze its natural substrate, laminarin. The purified recombinant *Hevea* β-glu was assayed for its antifungal activity against *Phytophthora*. When the fungus was grown on PDA plates, clear inhibition zones were observed around the filter paper discs soaked with 10 µg of the purified protein, indicating the inhibitory action of the purified enzyme.

Keywords: Antifungal protein, β-1,3-glucanase, *Hevea*, Recombinant protein

INTRODUCTION

Disease resistance in plants is brought about by constitutive and induced mechanisms. Accumulation of pathogenesis-related (PR) proteins is one of the most common markers for active, induced plant defense. β-1, 3-glucanases (β-glu) are considered to be one of the major components of this broad, generalized defense mechanism of plants against pathogen attack and are classified as PR-2 proteins. These are abundant, highly regulated hydrolytic enzymes widely distributed in the plant kingdom. β-glu have been studied in detail in different plant species and their role in combating the invading pathogens has been well documented in many incompatible plant-pathogen interactions (Simmons, 1994;

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