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SEQUENCE CHARACTERISATION OF β–1, 3-GLUCANASE GENE FROM *HEVEA BRASILIENSIS* THROUGH GENOMIC AND cDNA CLONING

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Accumulation of different isoforms of β–1,3-glucanase gene has been reported in various plant species in response to pathogen infection and other forms of stress. Although their major role is in disease resistance, they are also involved in various developmental and physiological processes. The genomic and cDNA sequences encoding β–1,3-glucanase gene in *Hevea brasiliensis* were amplified with gene specific primers, which were designed based on the available cDNA sequence and conserved amino acid domains of different plant β–1,3-glucanases. Under optimal PCR conditions a 968 bp DNA fragment was amplified from genomic DNA. Reverse transcription and amplification of the cDNA also yielded a similar 968 bp fragment. These bands were cloned and sequenced. Both PCR and RT-PCR products were the same and showed homology with the previously reported sequences. No intron was present in the coding region of 316 amino acid final functional protein. Southern hybridisation confirmed the presence of a low copy number gene with no difference among the *Phytophthora* tolerant and susceptible *H. brasiliensis* clones studied. Northern hybridisation and RT-PCR analysis showed higher expression of β–1,3-glucanase in latex than in leaves. The possible roles of β–1,3-glucanase gene in combating the abnormal leaf fall disease in *Hevea* and its likely involvement in somatic embryogenesis are also discussed.

Key words: β–1,3-glucanase, cDNA cloning, Gene amplification, Gene expression, *Hevea brasiliensis*.

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INTRODUCTION

The β–1,3-glucanases (PR-2 proteins) have been well characterized in different plant species (Castresana *et al.*, 1990; Beerhues and Kombrink, 1994; Pneumans *et al.*, 2000). They exist as multiple structural isoforms that differ in size, isoelectric point, primary structure, cellular localization and pattern of regulation (Meins *et al.*, 1992) and are subdivided into different classes. The Class I and Class II enzymes are pathogen inducible basic proteins, the former accumulating predominantly in the vacuoles (Keefe *et al.*, 1990) and the latter in the extra cellular compartments (Ward *et al.*, 1991). An acidic extra cellular PR-2d protein induced during infection is classified as Class III, as it differs at least by 43% from Class I and Class II enzymes (Payne *et al.*, 1991). The Class IV isoforms of β–1,3-glucanase are non-responsive to pathogen infection (Van Eldick *et al.*, 1996).

Of the several important roles suggested for β–1,3-glucanases in plants, their possible role in defense against invading pathogens evokes major attention. Accumulation of different isoforms of β–1,3-glucanase has been reported in various plant species in response to pathogen infection, or by other forms of stress, such as treatment with elicitors, ethylene, hormones, chemicals or heavy metals as well as by wounding, low temperature, ozone and UV light (Thalmair *et al.*, 1996; Hincha *et al.*, 1997; Brederode *et al.*, 1991). Plants have developed a variety of constitutive and inducible mechanisms to resist the colonization of a potential pathogen. Induction and accumulation of PR-proteins including β–1,3-glucanases in the infected tissue was often observed in incompatible plant-pathogen interactions (Egea *et al.*, 1999; Philip *et al.*, 2001; Jebakumar *et al.*, 2001). β–1,3-Glucanase catalyzes the