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ORIGINAL ARTICLE

Identification and cloning of class II and III chitinases from alkaline floral nectar of *Rhododendron irroratum*, Ericaceae

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Abstract

Main conclusion **Class II and III chitinases belonging to different glycoside hydrolase families were major nectarins in *Rhododendron irroratum* floral nectar which showed significant chitinolytic activity.**

Previous studies have demonstrated antimicrobial activity in plant floral nectar, but the molecular basis for the mechanism is still poorly understood. Two chitinases, class II (Rhchi2) and III (Rhchi3), were characterized from alkaline *Rhododendron irroratum* nectar by both SDS-PAGE and mass spectrometry. Rhchi2 (27 kDa) and Rhchi3 (29 kDa) are glycoside hydrolases (family 19 and 18) with theoretical pI of 8.19 and 7.04. The expression patterns of *Rhchi2* and *Rhchi3* were analyzed by semi-quantitative RT-PCR. *Rhchi2* is expressed in flowers (corolla nectar pouches) and leaves while *Rhchi3* is expressed in flowers. Chitinase in concentrated protein and fresh nectar samples was visualised by SDS-PAGE and chitinolytic activity in fresh nectar was determined spectrophotometrically via chitin-azure. Full length gene sequences were cloned with Tail-PCR and RACE. The amino acid sequence deduced from the coding region for these proteins showed high identity with known chitinases and predicted to be located in extracellular space. Fresh *R. irroratum* floral nectar showed significant chitinolytic activity. Our results demonstrate that class III chitinase (GH 18 family) also exists in floral nectar. The functional relationship between class II and III chitinases and the role of these pathogenesis-related proteins in antimicrobial activity in nectar is suggested.

Keywords Alkaline nectar · Chitinolytic activity · Class II chitinase · Class III chitinase · Glycoside hydrolase · Pathogenesis-related proteins

Abbreviation

GH Glycosyl hydrolase

Introduction

Floral nectar is a rich source of sugar, amino acids, vitamins, organic acids, metal ions, and other metabolic components, which makes it potentially an excellent microbial growth medium (Nicolson and Thornburg 2007). Insects that visit flowers are non-sterile (Evans and Armstrong 2006), therefore as well as pollen, they can also transfer between flowers any microorganisms that they carry (Ferrari et al. 2006). Furthermore, flowers can remain open for several days, during which time their metabolically rich nectar would potentially allow microbial growth in close proximity to the plant's reproductive tract. However, despite this, infections of the gynoecium are relatively rare in plants. This implies

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