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ORIGINAL PAPER

Antimicrobial peptide (AMP) from *Zingiber zerumbet* rhizomes with inhibitory effect on *Pythium myriotylum* secretory proteases and zoospore viability

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Abstract
Protease mediated proteolysis has been widely implicated in virulence of necrotrophic fungal pathogens. This is counteracted in plants by evolving new and effective antimicrobial peptides (AMP) that constitute important components of innate immune system. Peptide extraction from rhizome of *Zingiber zerumbet* was optimized using ammonium sulphate (50–80% w/v) and acetone (60 and 100% v/v) with maximal protein recovery of 1.2 ± 0.4 mg/g obtained using 100% acetone. Evaluation of inhibitory potential of *Z. zerumbet* rhizome protein extract to prominent hydrolases of necrotrophic *Pythium myriotylum* revealed maximal inhibition of proteases (75.8%) compared to other hydrolytic enzymes. Protein was purified by Sephacryl S200HR resin resulting in twofold purification and protease inhibition of 84.4%. Non-reducing polyacrylamide gel electrophoresis (PAGE) of the fractions yielded two bands of 75 kDa and 25 kDa molecular size. Peptide mass fingerprint of the protein bands using matrix assisted laser desorption/ionization (MALDI)-time of flight (TOF) mass spectroscopy (MS) and subsequent MASCOT searches revealed peptide match to methylesterase from *Arabidopsis thaliana* (15%) and to hypothetical protein from *Oryza sativa* (98%) respectively. Further centrifugal filter purification using Amicon Ultra (10,000 MW cut-off) filter, yielded a prominent band of 25 kDa size. Concentration dependent inhibition of zoospore viability by *Z. zerumbet* AMP designated as *Zz*AMP was observed with maximal inhibition of 89.5% at 4 µg protein and an IC_{50} value of 0.59 µg. Studies are of particular relevance in the context of identifying the molecules involved in imparting below ground defense in *Z. zerumbet* as well in development of AMPs as potential candidate molecules for control of necrotrophic pathogens of agricultural relevance.

Keywords Antimicrobial peptide · *Zingiber zerumbet* · *Pythium myriotylum* · Protease inhibitor · Zoospore

Introduction

Necrotrophic soil-borne pathogens that cause root and stem rots pose a serious problem to many agriculturally important plants. Based on host specificity, necrotrophs can be categorized as host-specific and broad host-range necrotrophs (Wen 2013). While the former has limited host range, infecting single or few related plant species, the latter group can infect different plant families (Radwan et al. 2014; Laluk and Mengiste 2010). Necrotrophs are known to secrete a substantial array of hydrolases or cell-wall degrading enzymes (CWDEs) for extensive plant tissue maceration to gain nutrition (Kubicek et al. 2014). In fact, secretion of such diverse array of hydrolytic enzymes enables necrotrophs with broad host-range to invade various agronomic crops with different cell wall compositions (Laluk and Mengiste 2010). Considering the fact that broad host-range necrotrophs pose major threat to crop productivity, experiments have been carried out by various groups to elucidate the defense response against broad host-range necrotrophs in model plants (Liang and Rollins 2018).While many important components of plant defense responses have been elucidated in model plant species, these are not archetypal as they do not replicate the biology of all plants owing to species-specific features (Freeman and Beattie 2008; Berni et al. 2018). Wild plants are usually well adapted to

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