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Plant Defensins-Its Antifungal Action and Applications

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Plant defensins are cysteine-rich antimicrobial peptides (AMP), which are tiny, extremely persistent molecules that make up the innate immune system and have antimicrobial effects against microbes as well as molecular aspects on their mode of action against bacteria and fungi. After comparing a new family of plant antifungal peptides with established insect defensins, Broekaert developed the term plant defensin. Plant defensins have also been studied as biotechnological techniques for improving agricultural productivity by generating fungus resistance in genetically modified organisms (GMOs). Transgenic plants that express defensins are highly resistant to fungi. According to current research, some plant defensins are not only harmful to microorganisms but also play a function in the growth and development of plants.

Key words: Antimicrobial peptides; Biotechnological tools; Genetically modified organisms; Plant defensins; Transgenics.

Introduction

Plants are extremely vulnerable to a variety of diseases and pests in the natural world; to limit this vulnerability, plants must evolve intricate defence mechanisms to defend themselves from these pathogens and pests. For the formation of these defence mechanism, several defence components are produced, which includes hydrogen peroxide (H_2O_2), terpenoids, polyacetylenes, phenolics, alkaloids and a broad spectrum of pathogenesis-related (PR) defence proteins and plant defensins.

Defensins are different components of a majority family of cationic host defence peptides (HDP) which are present in both the plant and animal domains. Defensins and defensin-like peptides have a wide range of functions, including membrane disruption and functioning as antagonists in order to identify and indicate cells. In the early years of the 1990s, the first members of the plant defensin family have been found in wheat and barley grains. They have been found to be identical to thionins in size (5 kDa, 45 to 54 amino acids) and cysteine content (typically 4, 6 or 8 cysteine residues), these protein molecules were initially dubbed thionins. More than 80 distinct plant defensin genome sequences from various plant species were analysed. A search of the UniProt database reveals 371 plant defensin papers that are now available for study. The *Arabidopsis* genome alone comprises around 300 defensin-like peptides (DEFL), 78% of which feature a cysteine-stabilized-helix-sheet (CS) pattern shared by plant and invertebrate defensins. Furthermore, plant EST programmes have uncovered over 1,000 DEFL genes.

Plant defensins, with a few significant exceptions, lack antibacterial action, in contrast to insect and mammalian defensins, which are largely antibacterial in nature. The vast majority of plant defensins play a role in defence against various fungi. They are effective not just against human pathogenic fungi like *Candida albicans* but also against baker's yeast

(*Saccharomyces* spp.) and phytopathogenic organisms like *Fusarium culmorum* and *Botrytis cinerea*. In *Arabidopsis thaliana*, it has also been demonstrated that plant defensins can influence the growth of many tomato plant parts, which serve various defensive and developmental purposes (Hegedus and Marx, 2013).

Plant defence proteins in *Arabidopsis thaliana* can affect the development of several parts of tomato plant, which have a variety of defensive and physiological functions of growing seedlings and germinating seeds. Plant defensins are found in the xylem, stomata, stomata cells, parenchyma cells and other auxiliary tissues. When it is thought that these sites represent the first points of contact with a possible pathogen, the existence of such peptides throughout the various tissues is consistent with a defensive role for them. Defensin proteins are clearly implicated in plant defence as evidenced by a number of characteristics. Their distribution is in line with their alleged defensive function. They have been found in leaves, tubers, flowers, pods and seeds, and they are known to be critical to germinating seeds.

Antifungal Activity

The majority of plant defensins have two classes of mature transcripts. The primary and biggest form of source protein is composed of an amino-signal peptide that transmits the peptide to the extrinsic region. The second family of defensins evolved from larger precursors with C-terminal prodomains. Plant defensin genomes from diverse plant species were analysed, and it was discovered that only eight cysteine, two glycine and one glutamate acid residues shows any sort of significant structural conservation. The bulk of plant defensins have a strong antifungal activity, with the exception of those that exhibit antibacterial or insecticidal activity. Antimicrobial categories typically have unique antifungal properties, such as those that change the intended fungal hyphae's shape to hinder their growth and cellular entry (Lacerda *et al.*, 2014). Plant defensins antifungal activity is governed by structural factors, although it is yet unknown how exactly they stop fungal development at anatomical levels.

Plant defensins are unable to communicate directly with cell membrane phospholipids, in contrast to defensins from mammals and insects. In sensitive yeast and fungus, RsAFP2 reacts with glucosylceramides (GlcCer) in the membranes and causes membrane permeability and fungal cell lysis. According to Aerts *et al.* (2007), the RsAFP2 involves in the generation of reactive oxygen species (ROS) in *Candida albicans*, which causes the membrane to become permeable and stops the growth of the fungus. Defensins can change calcium signalling, which is crucial for the growth of fungal hyphal tips. For instance, the lucerne defensin (MsDef1) can limit hyphal development and cause fungal hyphae to hyper branch by blocking the L-type Ca^{2+} channel. In addition to their biological activities, some plant defensins exhibit proteinase, α-amylase and protein translation inhibition, which may support their defense-related functions.

Biotechnological Applications and Transgeny

There are numerous transgenic plants on the market with extra genes that code for proteins that confer resistance to herbicides and insect pests, but no transgenic plant is yet available that has been proven resistant to phytopathogenic fungus or even contains plant defensins as the resistant component. But several investigations have shown that antifungal defensins have effective antifungal activity when incorporated into various host plants. Plant defensins exhibiting antifungal properties have thus emerged as the initial chemicals that can be used to create genetically engineered crops with phytopathogenic resistance.

The first examination of transgenic plants containing foreign antifungal defensin genes was performed in tobacco plants encoding Rs-AFP2, a protein obtained from radish. The altered tobacco plants had elevated levels of peptide translation and increased resistance to the phytopathogenic fungus *Alternaria longipes*. Four years later, the same defensin was tested against dangerous fungus species in tests with transgenic apple plants. Transgenic plants were chosen as a result of the *Agrobacterium tumefaciens* modification and the peptide was extracted and examined (Stotz *et al.*, 2009). *In vitro* trials showed that synthesised peptide could suppress the germination of *Fusarium culmorum* spores. Tomato genotypes were also modified with Rs-AFP2, which increased the antifungal properties. The leaves of tomato plants overexpressing the radish defensin were isolated and examined for antifungal activity against *Alternaria solani*, *Fusarium oxysporum* and *Rhizoctonia solani*. A crude extract of tomato leaves expressing radish defensin was demonstrated to prevent the action of all of the fungi mentioned above. Furthermore, in 2002, Rs-AFP2 was tried in genetically modified crops again, this time with two pear cultivars, Burakovka and Pamyat Yakoyleva. The leaves of pear plants were collected after transformation for PCR and western blot hybridization experiments. The presence of the alien gene and the recombinant peptide was validated using appropriate techniques, confirming plant transformation success. However, the antifungal effectiveness of GMO pear plants expressing Rs-AFP2 against pathogenic fungi needs to be evaluated *in vitro* and *in vivo*. Jha *et al.* reported the most recent investigation on Rs-AFP2 in 2009, when they converted this peptide into rice (*Oryza sativa* L. cv. Pusa Basmati). The mutant plants were evaluated *in vitro* and *in vivo* against *Magnaporthe oryzae* and *Rhizoctonia solani*, the two most common causes of rice losses in agriculture, demonstrating that Rs-AFP2 overexpression can protect against rice blast and sheath blight pathogens respectively. Additional study on genetically modified plants producing an antifungal defensin has been reported. As a consequence, it became clear that pea defensins introduced into *Brassica napus* cultivars improved their resistance to *Leptosphaeria maculans*, the causative agent of plant blackleg. Tobacco plants were also modified using *Agrobacterium tumefaciens* and an antifungal gene was generated. Tobacco resistance to *Aspergillus flavus* and *Verticillium dahlia* has been enhanced by the translated peptide, D4E1. Tobacco was also utilised to modify the stamen defensin BSD1 and the resulting peptide provided the plant with increased resistance to the harm caused by *Phytophthora parasitica*. Transgenic plants showed enhanced resistance to *Fusarium moniliforme* and *Phytophthora parasitica* after being modified with the mustard defensin – BjD. ZmDEF1, a maize defensin, was recently introduced into tobacco plants and showed increased resistance to *Phytophthora parasitica* (Wang *et al.*, 2011). Transgenic groundnut plants showing the same mustard defensin showed increased resistance to late leaf spot pathogens *Cercospora arachidicola* and *Pheaoisariopsis personata* (Anuradha *et al.*, 2008).

Conclusion

Plant defensins pertain to a world of defence mechanism opportunities and new peptides with various actions have yet to be identified, as well as investigations with thousands of plant species. Numerous peptides have previously demonstrated significant activity against such infections, with great promise for use in the creation of a commercial fungicide or incorporation into transgenic plants. It is also envisaged that in the near future, commercial agro-products made from antifungal defensins would be targeted as crucial for increasing crop yield. This will promote and hasten the shift from biotechnological research to bioproduct field application.

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