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Protein-based products as resistance inducers: disease control and mechanisms of action

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Highlights

- Leaf treatments with a protein derivative represent a sustainable strategy in plant protection, because they induce grapevine resistance, and change the structure of leaf microbial communities on grapevine.
- Plant-protein hydrolysates reduce powdery mildew severity, and their biocontrol activity is affected by the protein source, degree of hydrolysis and peptide composition.

Introduction

Grapevine (*Vitis vinifera*) is one of the major fruit crops in the world, and downy mildew (caused by the oomycete *Plasmopara viticola*) is a serious disease that requires frequent fungicide applications. Increasing concerns about the negative impacts of pesticides on human health and the environment encourage the development of harmless alternatives to synthetic chemicals, such as resistance inducers (Delaunois *et al*., 2014). Proteins and peptides represent a wide category of plant elicitors (Albert, 2013), and the protein derivative called Nutrient Broth (NB) showed a high efficacy in controlling powdery mildew under field conditions (Nesler *et al*., 2015). This study aimed to dissect the mechanisms of action of NB against grapevine downy mildew caused by the oomycete *P. viticola* and to develop low-cost protein hydrolysates from agro-industrial by-products.

Material and methods

Grapevine plants (Pinot noir ENTAV115) grown under greenhouse conditions or *in vitro* (Nesler *et al*., 2015) were kept untreated (UNT) or treated with water (H_2O), 3.0 g/l NB (Nesler *et al*., 2015), or with a commercial product based on laminarins (LAM, 0.75 ml/l Vacciplant, Belchim Crop Protection). RNA extraction and quantitative real-time PCR reactions were carried out for the amplification of pathogenesis-related genes (*PR-1*, *PR-2*, and *PR-4*), osmotins (*OSM-1* and *OSM-2*) and chitinase (*CHIT-3*) (Nesler *et al*., 2015).

Collection of phyllosphere microorganisms, DNA extraction and amplification of bacterial (V6-V8 of the 16S rRNA) and fungal (ITS3-ITS4 of the internal transcribed spacer, ITS) fragments were performed as described by Cappelletti *et al*. (2016).

Soybean, rapeseed and guar meals were subjected to enzymatic (Alcalase or Flavourzyme at 1% or 50% E/S) or chemical (6 N sulfuric acid, H_2SO_4; condition A: 121 °C, 15 min, condition B: 100 °C, 8 h) hydrolysis (Cappelletti *et al*., 2017). Courgettes (*Cucurbita pepo*) and powdery mildew caused by *Podosphaera xanthii* were selected as easy-to-handle study pathosystem. Courgette plants (cv Nero Milano) grown in greenhouse (Nesler *et al*., 2015) were sprayed with protein hydrolysates (1 g/l), water (H_2O) or non-hydrolysed protein sources (N-H), and for the acid hydrolysis with a potassium sulfate (K_2SO_4) solution. The identification of peptides and amino acids was performed by an external service company (ISB Srl, Italy).

Results and discussion

The preventive foliar application of NB reduced downy mildew severity as compared with control plants (UNT and H_2O-treated), and the efficacy was higher in NB- than in LAM-treated plants. The expression levels of *PR-1*, *PR-2*, *PR-4*, *OSM-1*, *OSM-2* and *CHIT-3* genes were upregulated by NB before *P. viticola* inoculation, demonstrating the induction of grapevine resistance. Although the expression level of *CHIT-3*, *OSM-1*, *OSM-2* and *PR-4* was higher in LAM- as compared with NB-treated plants, LAM showed lower efficacy than NB against downy mildew, suggesting that multiple mechanisms of action are involved in the biocontrol activity of NB.

Indeed, NB changed the structure of phyllosphere bacterial and fungal populations as compared with control plants (UNT and H_2O-treated), and these modifications were affected by the composition of the originally residing microbiome. The NB treatment increased the proportion of some genera (e.g. *Exiguobacterium*, *Pseudomonas*, *Serratia*, *Lysobacter*) that potentially include biocontrol strains, suggesting that these changes may contribute to disease control. Furthermore, experiments using *in vitro* grown plants, in the absence of phyllosphere microorganisms, showed that the NB reduced downy mildew symptoms as compared with H_2O-treated plants, and induced the expression of *PR-2*, *PR-4*, *CHIT-3*, *OSM-1* and *OSM-2* before *P. viticola* inoculation. In conclusion, NB reduced downy mildew symptoms mainly by the induction of defence mechanisms in grapevine, and changed proportions of some microbial taxa linked to the biological control of plant pathogens, possibly providing a partial contribution to the control of downy mildew and to the activation of defence signalling pathways.

In order to develop cheaper and environmental-friendly protein-based products to control grapevine diseases, courgette powdery mildew was used as preliminary model pathosystem. Protein hydrolysates obtained by agro-industrial by-products were obtained, and guar hydrolysates significantly reduced powdery mildew symptoms. Particularly, two specific hydrolysis methods led to the formation of bioactive products (guar enzymatic hydrolysate Alcalase 50% and guar acid hydrolysate condition B). The biocontrol activity of hydrolysates was affected by the original protein source, the method and the degree of hydrolysis, namely the percentage of cleaved peptide bonds. The composition in free amino acids and peptide fragment could regulate plant responses to the pathogen infection. However, the use of strong acids during the hydrolysis causes an increase of salinity (K_2SO_4) of protein hydrolysates, which contributes to the disease control. The foliar application of low-cost protein hydrolysates represents an innovative approach to control crop diseases, and further studies are required to fully clarify their mechanisms of action and the effects on phyllosphere microorganisms.

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