

Phosphoproteomics Analysis of MAMPs Signalling in Plant Immunity

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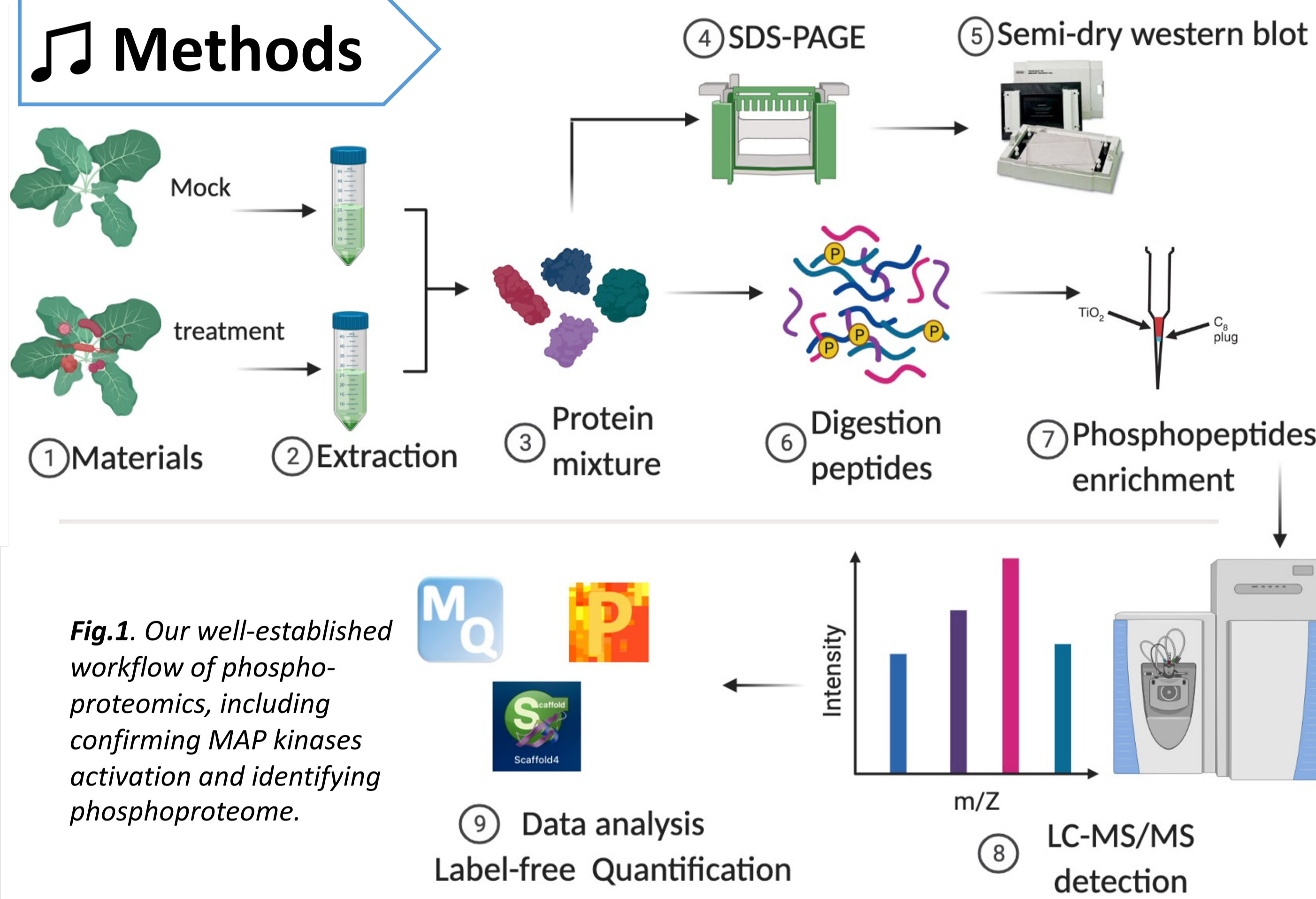
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Introduction

Vegetable Brassicas are important food crops but suffering from various pests and diseases. The first layer in plant immunity are called Microbe-Associated Molecular Patterns (MAMPs). These MAMP signaling pathways have been widely studied in the model plant *Arabidopsis thaliana*, but the overlap and differences among these mechanisms remain unclear in crop plants such as *Brassica oleracea*. Phosphorylation is an excellent post-translational modification to focus on because it can form the basis for physical enrichment of signal-transduction components and could be identified by high-throughput Mass spectrometry.

Aim: to identify defence genes and active immune pathways that can be used to breed Brassica vegetables.

Methods



Results

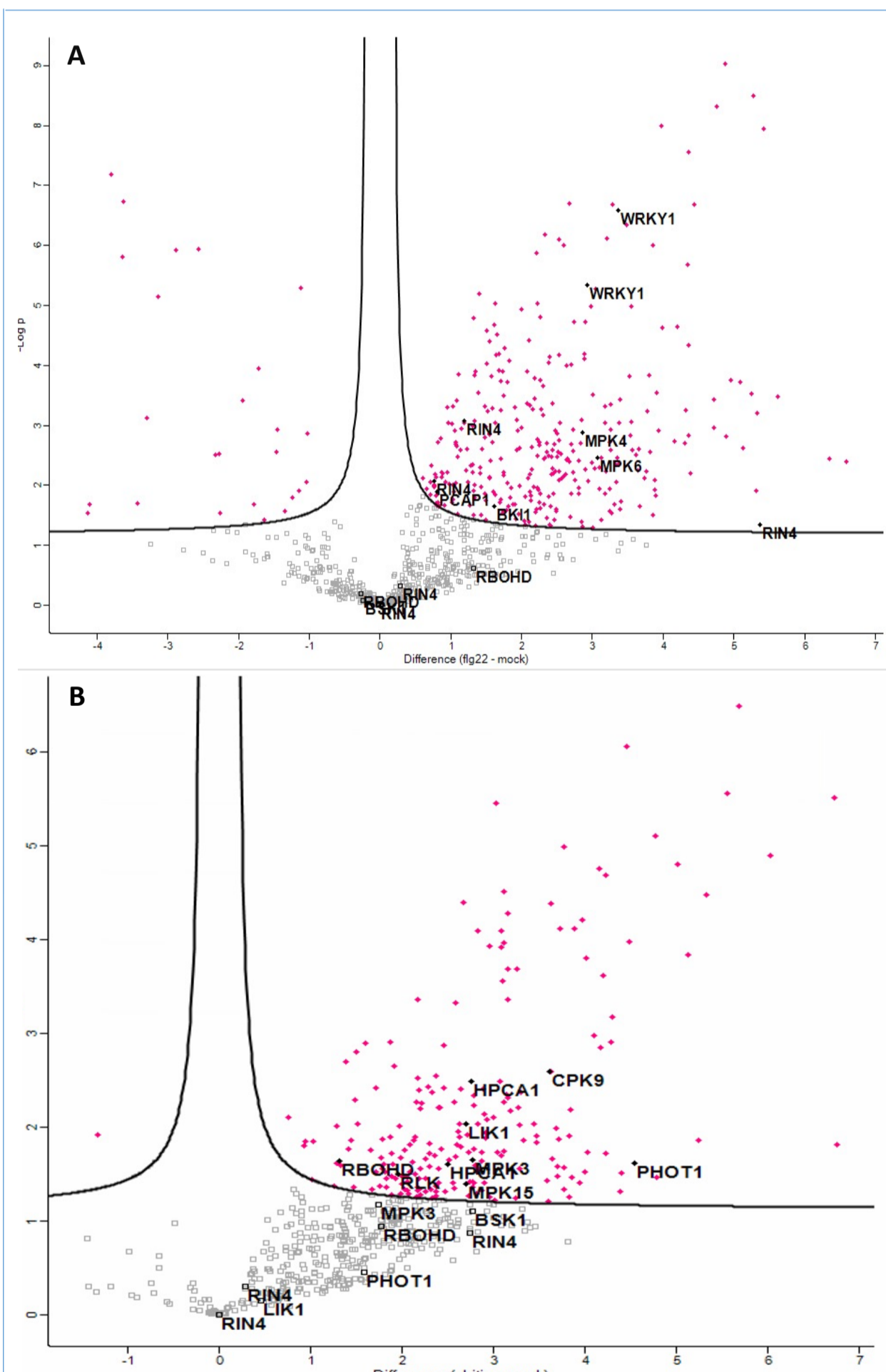
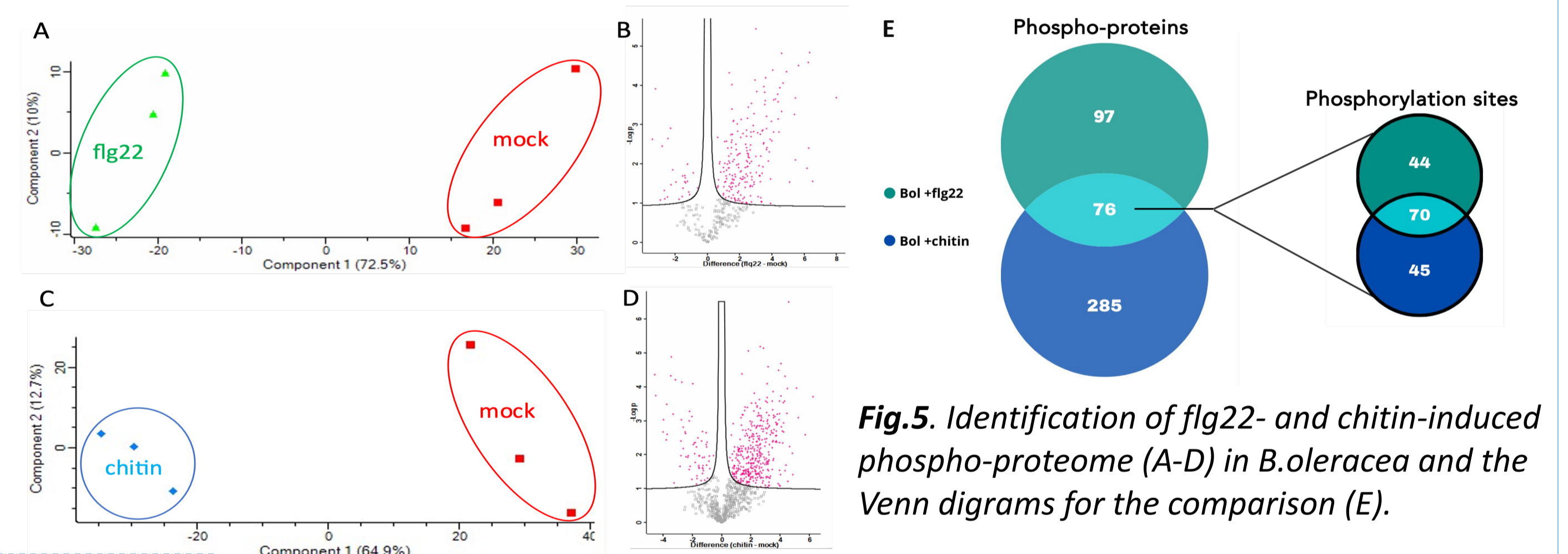
Transient activation of MAPKs

The kinetics of MAP kinase activation in seedlings of *A.thaliana* (Col-0) and *B.oleracea* (DH1012) treated with either 1 μ M flg22 or 100 μ g/ml chitin are shown by immunoblot analysis using an anti-p44/42-ERK antibody.

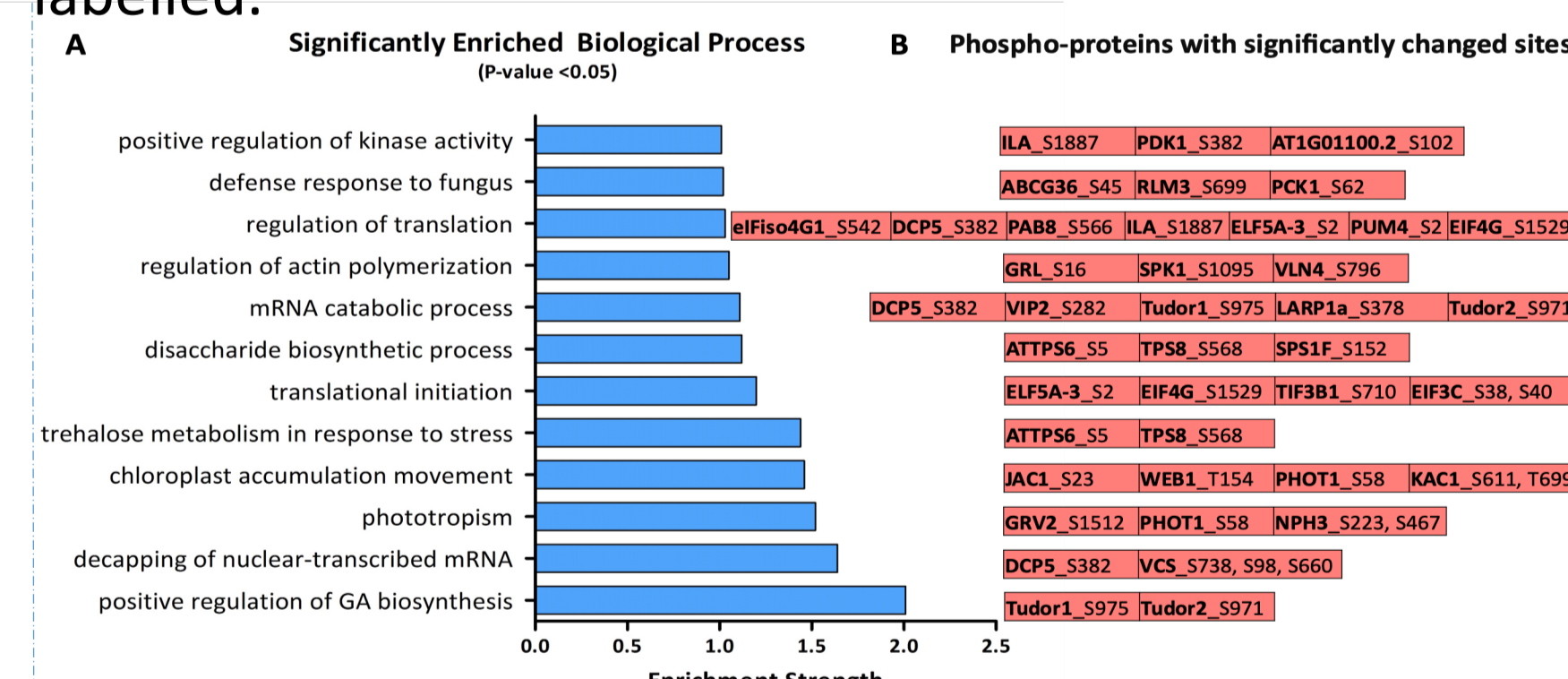
Fig.2. Time course of MAPKs activation in *Arabidopsis* (A) and *Brassica* (B)

In the case of loading the same amount of total protein, the strongest MAPK activation by flg22 in Col-0 (Fig.2A) is 15 min and 5 minutes later by chitin. As for DH1012 (Fig.2B), MAPKs phosphorylation always peaks at 15 min with flg22 or chitin treatment.

flg22 and chitin-induced phosphoproteomic profile in Col-0 and DH1012

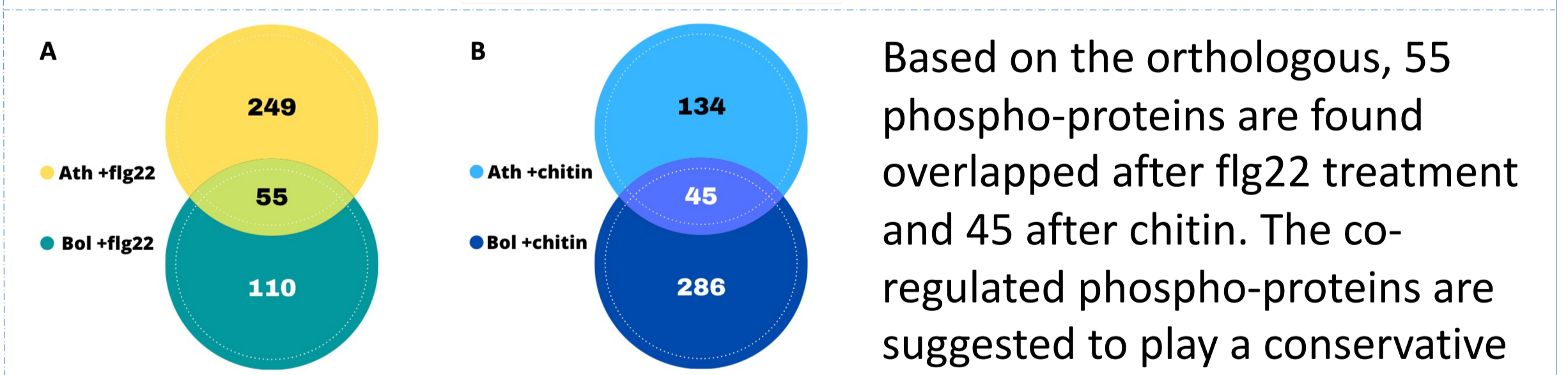


The flg22-induced immunity in *Arabidopsis* is a classical model and many important components have been well reported as well. Our data supported that some of these well-known players significantly changed in the phosphorylation level with flg22 treatment (Fig.3A, red dots). The significantly changed phosphorylation sites induced by chitin treatment (Fig.3B) and some of the well-known proteins identified in our dataset are labelled.



Processes in response to stress and fungus are enriched as expected and there might be a closely crosstalk between chitin-triggered immunity and GA hormone signaling.

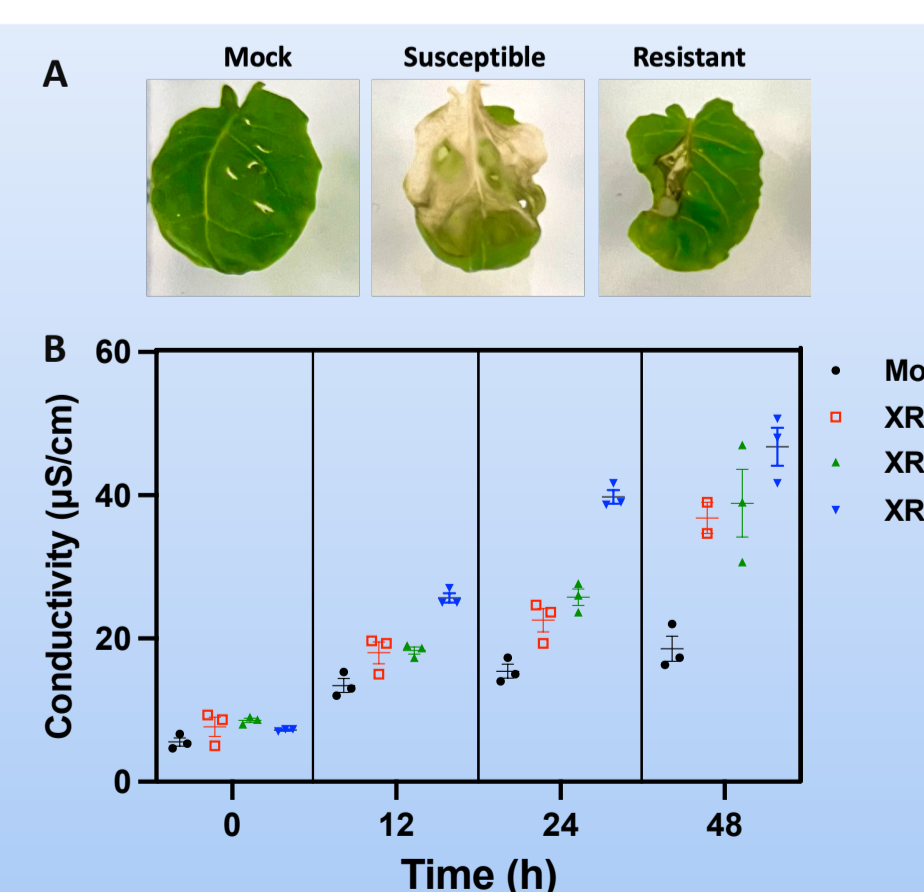
Principal component analysis (A,C) and volcano plots (B,D) show the statistical analysis of Brassica datasets. Venn Diagrams of the two datasets comparison show that 76 phospho-proteins overlapped might participate in the two both kinds of PAMPs-triggered immunity pathways.



Based on the orthologous, 55 phospho-proteins are found overlapped after flg22 treatment and 45 after chitin. The co-regulated phospho-proteins are suggested to play a conservative role in flg22 or chitin signalling between different species.

Conclusion

- An effective phospho-proteomics experiment system has been established.
- The transient of MAP kinases activation have been seen in both *Arabidopsis* and *Brassica* seedlings when treated with flg22 or chitin.
- These well-known players in the flg22 signalling of *Arabidopsis* have been identified in our datasets as well and some of the phospho-sites are newly found here.
- The overlap and differences between flg22 and chitin signaling in *Arabidopsis* and *Brassica* really indicate the conservation and specialization between different immunity molecular patterns.



To better understand the immunity mechanisms in B.oleracea

- Examine a real disease interaction, Black rot
Which is caused by the bacterium *Xanthomonas campestris pv. campestris* (Xcc), one of the most severe diseases that affects cruciferous crops.
- Preliminary work has screened that DH1012 is susceptible to Xcc race 1 but resistant to 5 and 6 (Fig.7A). The ion leakage (Fig.7B) showed that the response from race 6 is significantly higher than the others as well.
- Thus, we are taking advantage of phospho-proteomics to study on the comprehension of resistance mechanisms and offer more candidate defence genes in genetic breeding.

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Future work



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