Strategies for Mapping of Autophosphorylation Sites in Plant Kinases Receptors.

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Introduction: Phosphorylation is an important mechanism of regulation of the enzyme activity and is involved in the transduction of cellular signals. Many plant kinases receptors are phosphorylated in response to extracellular signals related to regulatory cascades, such as infections by pathogens. The sites and the level of phosphorylation will determinate the pattern of this response. Thus, the identification of the phosphorylation sites have been essential in the elucidating of the signal transduction mechanisms in plant receptors. However, the determination of the phosphorylation sites is challenging. **Objectives**: In this work, we present a strategy for mapping auto-phosphorylation sites by mass spectrometry. **Methods**: In the first stage, we applied tryptic mass fingerprinting technique by MALDI TOF/TOF to identify possible phosphorylated ions, followed by confirmation by ION TRAP MS, using the *multiple reaction monitoring* (MRM) approach, combined with *de novo sequencing* and neutral loss. **Results**: Applying this strategy, we identified 6 sites in the NIK (NSP-Interacting kinase) and 12 sites in the BR-1 (brassinosteroid-Insensitive-1). **Conclusion**: Our strategy showed to be efficient in the identification of phosphorylation sites in plant kinases.