A new protease-trap based on cacao recombinant protease inhibitor immobilized in activated cnbr-sepharose.

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The activity of many proteins, such as transcription factors, kinases, and proteases, depends on posttranslational modifications that frequently are not detected by transcriptomic and proteomic technologies. Phytocystatins are cysteine-proteinase inhibitors from plants implicated in several physiologic processes. We identified four cystatins ORFs (*Tc*CYS1, 2, 3 and 4) using the data from two cDNA libraries corresponding to resistant and susceptible interactions between *T. cacao* and *Moniliophthora perniciosa*. *Tc*CYS1 and 4 are proteins with about 23-24 kDA that show an extended carboxy segment contain SNSL sequence that function with legumin inhibitor site. These ORFs and truncated forms contain carboxy segment were sub cloned, and His-Tag fused proteins expressed in *E.coli* using pET28a vector. To monitor activity of proteases rather than their abundance, we introduce a new protease activity profiling in plants. This technology is based on the use of recombinant protease inhibitor (cystatin), reversible protease inhibitors that react with active proteases in a mechanism-based manner. The recombinant cystatins were coupled to CNBr-activated sepharose. The proteases capture was performed by resin incubation at 37^{o}C for 30 min, under agitation, with 10 volumes of 1 mg/mL of proteins from *M. perniciosa* culture supernatant or cacao tissue extracts. The bound proteases were eluted with 50 mM glycine pH 2.9, for 5 min, and then equilibrated with equal volume of 10 mM Tris-Base. For qualitative analysis of captured proteases, 0.1% gelatin/SDS-PAGE was used. Two neutral protease isoforms were captured in *M. perniciosa* culture supernatant and several acidic isoforms were captured from cacao tissues by immobilized cystatins. Truncated recombinant carboxy segment did not show inhibitory activity against papain, but captured one protease isoform from cacao tissues. These results demonstrate that this technology can identify differentially activated proteases and/or characterize the activity of a particular protease within complex mixtures.
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