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SHORT COMMUNICATION

## Detection of novel trypsin inhibitors in the cotyledons of *Phaseolus vulgaris* seeds

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### ABSTRACT

Protease inhibitors play important roles in plants in association with stress. Trypsin inhibitors (TIs) in particular are known to act as protective agents against insect and pathogen attacks. The growing relevance of these inhibitors requires expedited techniques for their detection. By using the two-dimensional electrophoresis (2-DE) reverse zymography technique, we identified, from the crude extract of bean seeds, nine novel polypeptides that showed trypsin inhibitor activity. One of these polypeptide inhibitors yielded no homology in the database, which can be an indication that we are found a new protein with unique TI properties. The remaining showed homology with proteins annotated in the UniProt database and form, together with a Kunitz type inhibitor, a new TI cluster for *Phaseolus* spp. Three of these polypeptides showed additional high homology with lectins, likely indicating that they have lectin properties, while the other five showed high homology with  $\alpha$ -amylase inhibitors, indicating that they probably have a dual inhibitory effect against trypsin and the  $\alpha$ -amylase enzyme. These bifunctional inhibitors can be highly useful for crop management, since the two inhibitory activities are important for plants when coping with pathogen and pest attacks.

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Abbreviations: CHAPS, 3-[3-cholamidopropyl(dimethylammonio)]-1-propane-sulphonate; TIs, trypsin inhibitors; 2-DE, two-dimensional electrophoresis

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