UTILIZATION OF COLLOIDAL CHITIN STAINED WITH REMAZOL BRILLIANT BLUE R AS A SENSITIVE, RAPID AND ECONOMICAL ALTERNATIVE TO DETECT CHITINASE IN PLANTS

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Chitinases are enzymes found in several organisms and have been implicated in a wide range of biological events, including defense against pathogens, biological control, recycling of insect cuticle and cellular differentiation. When plants are challenged by pathogenic microorganisms, especially fungi and insects, the expression of many defensive genes, including those associated with chitinase synthesis is induced. Plant chitinase degrades the polymer N-acetylglucosamine present in the cell wall of pathogenic fungi and also in the cuticle of insects. Currently, commercial and conventional methods chitinase detection in plants involve the use of chitin in its solid state, stained with (Remazol Brilliant Blue R® - RBBR Sigma-Aldrich) as a substrate. These methods are expensive, slow and shows low sensitivity. This present study, proposes to assess chitinase in plants using chitin stained with RBBR in its colloidal state. Chitin in colloidal state was obtained using phosphoric acid 85%, pH 6.5 and humidity between 90 and 95%. Using sodium dichromate 1.5% commercially available and sodium potassium tartarate allowed the incorporation of RBBR. The colloidal chitin stained with RBBR (CC-RBBR) was boiled, strained, autoclaved and stored at 5°C. Commercially available chitinase of Streptomyces griseus and extracts of leaf tissue of bean and soy plants treated with resistance inducers were applied to evaluate the enzyme action on the substrates. The CC-RBBR presented two-to-three-fold higher sensitivity to the commercially available chitinase, as well as increased sensitivity for chitinase detection in proteic extracts of bean and soy. Results pointed out that the colloidal substract for detection of chitinase in plants presents high sensitivity and low cost, and demands little time to both prepare the assay and to interpret the data, establishing a viable alternative for the assessment of this enzyme in plants.

Keywords: Inducing Resistance in Plants, Phytopathogens, Substrates for Chitinase.